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Solid Phase Microextraction and Stir Bar Sorptive Extraction Coupled to Ion Mobility Spectrometry

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By

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DISSERTATION

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We certify that we have read this dissertation and that in our opinion it is adequate in scientific scope and quality as a dissertation for the degree of Doctor of Philosophy.

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Abstract

Ion mobility spectrometry (IMS) has received renewed interest in the last few years where only a decade ago the technique was regarded largely as a curiosity or an outdated technology. The resurgence of IMS instrumentation and technique is related to its intrinsic features of response and to practical considerations when compared with well-established technologies such as GC and GC/MS.

The success of solid phase micro extraction (SPME) and stir bar sorptive extraction (SBSE) in providing many advantages over conventional analytical methods has led us to further explore the techniques by coupling them to IMS to detect and quantify trace level of compounds. The extraction techniques provide enhanced sensitivity and analyte selectivity, which circumvents the problems of charge competition and ion suppression commonly found in IMS.

In the first part of the research, ephedrine is extracted from urine samples by SPME and vaporized into the IMS. The analytical procedure is optimized for various extraction paramaters and analyte desorption conditions. The carryover effects, peak shapes and drift times of ephedrine are also evaluated. The ion fragmentation pattern of ephedrine shows good comparison with chemical ionization mass spectra data. Good limits of detection and linearity are obtained showing that SPME-IMS compares well to other techniques for drug analysis.

In the second part of the research, the SPME-IMS technique is demonstrated for the detection and quantitation of 4-hydroxybenzoate preservatives, methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP) in pharmaceutical

products. For the first time, this technique is described for the simultaneous detection, separation and quantitation of multiple analytes in complex matrices. The four preservatives are separated in less than 18 ms. Analysis of real samples by SPME-IMS using benzyl paraben as an internal standard yields good comparison to an HPLC method, thereby reinforcing the applicability of this technique as a method for routine analysis.

In the final phase of the project, a newly developed SBSE-IMS technique is shown to be effective using thermal desorption for the trace detection of TNT and RDX. In addition to the successful development of the method in terms of extraction efficiency and ion separation; ions fragmentation patterns, carryover effects, drift times characteristics, and experimental parameters on the kinetics of thermal desorption are explored. The desorption kinetics is investigated using maximum desorption time, T_{Dmax}, and thermally optimized to prevent carryover. Limits of detection are determined to be 1.5 ng/mL for RDX and 0.1 ng/mL for TNT.

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Table of Contents

LIST OF FIGURES

LIST OF TABLES

HISTORICAL

Miniaturization has increasingly become a dominant trend in analytical chemistry, not only in instrumentation, but also in sample preparation. Typical examples of miniaturization in sample preparation techniques are micro-liquid–liquid extraction, disc–cartridge solid phase extraction (SPE), on-line SPE, liquid phase micro extraction (LPME), solid phase micro extraction (SPME) and stir-bar sorptive extraction (SBSE). In combination with state-of-the-art analytical instrumentation, the overall method can result in fast analysis, high sample throughput, low solvent consumption and little manpower per sample, with high sensitivity. In particular, the reduction of solvent consumption in analytical laboratories has an important effect on analytical costs.

A good analytical method involves sampling, sample preparation, separation, identification, and quantitation of target analytes in complex samples. Sample preparation is critical in any method because it is the step that isolates and purifies the analytes of interest in a form and concentration that can be readily analyzed. A successful sample preparation technique isolates the analyte of interest from as many matrix interferences as possible, dissolution or sorption of analytes in a suitable media and analyte pre-concentration. Ineffective sample preparation directly compromises the subsequent steps of separation, detection and quantitation of analytes.

Sample preparation steps may include clean up procedures, especially for very complex samples, such as biological or environmental samples. Before trace analyses can be determined in complex samples, they must first be isolated from the matrix, then concentrated, and often subjected to cleanups. In heterogeneous samples, mechanical

procedures such as filtration and centrifugation can be used to separate various phases. In a homogeneous mixture the separation of analytes must employ the differing physical and chemical properties of the individual components.

Although many instrumental techniques have evolved and matured into highly sensitive and automated devices, sample preparation is still considered to be slow, error-prone, labor-intensive and a bottleneck step in many analytical methods. Many analytical methods require as much as ten times as long to prepare the sample for analysis than to perform the actual analysis. Sample preparation also accounts for most of the errors encountered in an analytical method directly due to sample processing, operator error and contamination.

Many of the classical sample preparation methods have been available for decades with few improvements despite the increasing demands for fast, cost-effective, sensitive, automated, safe and generally more efficient methods. The ideal sample preparation technique would be the one that eliminates the use of toxic solvents, and offers simplicity, speed, automation, selectivity, sensitivity and error reduction. Practical demands for analysis of pharmaceutical products, the environmental studies, forensics, and life science are the main driving forces for development in sample preparation for spectroscopic and chromatographic analysis.

Liquid-liquid extraction (LLE) is one of the oldest sample preparation techniques still currently used. This is a separation process that takes advantage of the relative solubilities of solutes in immiscible solvents. The solute dissolves more readily and becomes more concentrated in the solvent in which it has a higher solubility. A partial separation occurs when a number of solutes have different relative solubilities in the two

solvents used. The benefit of LLE is that it can produce very clean extracts with good selectivity and sensitivity for targeted analytes.

LLE often requires large quantities of costly solvents that not only present a health hazard for chemists, but also cause disposal problems and environmental concerns. The emission of hazardous solvent vapors into the atmosphere during sample concentration also hinders the use of this technique for routine analysis. The LLE procedure is also not readily automated because some steps may lack continuity. The long time often required to perform liquid-liquid extractions and sample concentration adds to the inherent disadvantages of the technique. Renewed awareness of the pollution and hazards caused by many organic solvents has resulted in significant efforts to eliminate these substances and hence initiated a major change in sample preparation methodologies. Various other techniques such as headspace extraction, modified fluid and sorption extraction techniques have been developed for sample pretreatment in order to replace LLE or to introduce new approaches to sample preparation.

Headspace extraction is one of the methods that has evolved into a successful sampling technique currently in widespread use today in the environmental and pharmaceutical industry. The first analysis of the vapors above a liquid occurred in 1939 when Harger, Bridwell and Raney proposed the determination of the alcohol content of aqueous solutions [1]. The first reported use of static headspace with gas chromatography (GC) occurred in 1958 and the use of dynamic headspace, purge and trap, with GC, occurred in the 1970s [2]. Techniques for both static and dynamic headspace sampling in combination with GC have evolved significantly since the 1970's and the theory of headspace sampling and transfer of the samples to the GC has been well developed.

Headspace extraction is generally defined as a vapor-phase extraction, involving the partitioning of analytes between a non-volatile liquid or solid phase and the vapor phase above the liquid or solid. It is expected that the vapor-phase mixture contains fewer components than the usually complex liquid or solid sample and that this mixture is transferred to a gas chromatograph or other instrument for analysis. There are a number of techniques for sampling headspace vapors and introducing them to a gas chromatograph. Newer techniques have become miniaturized and automated.

Static headspace extraction has been widely used to test for volatile organic compounds (VOC) in pharmaceutical and environmental samples. It is most suited for the analysis of the very light volatiles in samples that can be efficiently partitioned into the headspace gas volume from the liquid or solid matrix sample. Higher boiling volatiles and semi-volatiles are not detectable with this technique due to their low partition coefficient in the vapor headspace volume. In addition, the technique lacks a concentrating effect and therefore exhibits low sensitivity, typically to concentrations in the ppm to ppt range. However, the technique is the preferred method for the analysis of gases and very light volatiles, which cannot be analyzed by other techniques. The technique is also preferred when sample automation is required such as in a quality control method or in sample screening.

In the 1980's Gangler et al presented the extraction of analytes from food and soil samples using microwave assisted solvent extraction (MAE) [3]. Since then, microwave digestion methods have been developed for different sample types such as environmental, biological, geological, and industrial applications.

Microwave-assisted extraction is a process of using microwave energy to heat solvents in contact with a sample in order to partition analytes from the sample matrix into the solvent. The ability to rapidly heat the sample solvent mixture is inherent to MAE and the main advantage of this technique. By using closed vessels the extraction can be performed at elevated temperatures accelerating the mass transfer of target compounds from the sample matrix. A typical extraction procedure takes 15–30 min and uses small solvent volumes in the range of 10–30 mL.

Today MAE equipment designed for laboratory purposes is safe to work with and offers the user various ways to control the extraction process. Commercial systems used for closed-vessel MAE consist of a magnetron tube, an oven where the extraction vessels are set upon a turntable, several monitoring devices for controlling the temperature and pressure, and a number of electronic components. Over the years procedures based on microwave ovens have replaced some of the conventional extraction techniques resulting in faster analysis and less solvent consumption [4].

In the past few decades various enhanced liquid extraction methods have been proposed to resolve the problems associated with classical solvent-based extraction techniques. Some of the sample preparation methods developed using less solvents are supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), microwave extraction, and enhanced solvent extraction (ESE). The most popular of these SFE, was introduced commercially in the early 1990's, and many laboratories replaced their conventional methodologies with new SFE methodologies to reduce solvent consumption and increase sample throughput [5].

Supercritical fluid extraction is the process of extracting analytes from complex matrices without use of harmful solvents. It typically utilizes the special properties of carbon dioxide or liquids such as isopropyl alcohol, which, when cooled and compressed, has the density of a liquid and the intermolecular forces of a gas. This is then able to penetrate the product and dissolve the oils or other components wanted. When the pressure is reduced, the solvent will then either evaporate harmlessly into the atmosphere, or be recovered and recompressed. The end result leaves only the concentrated extract. Although SFE has many advantages such as manifesting higher diffusion coefficients, achieve lower viscosities than conventional solvents, achieving fast kinetics and selectivity along with exhaustive extraction, the technique is still not widely popular in laboratories. SFE requires large and expensive instrumentation and large amounts of pure carbon dioxide. On site extraction using SFE can be too cumbersome and impractical for many applications.

The use of solid sorbents is one of the fastest developing fields in sample preparation. The property of solid surfaces to bind organic molecules via different affinity mechanisms has been known for over a hundred years. The possibility of using solid based sorbents to retain analytes was recognized when Tswett introduced chromatography in the early twentieth century. Initially drawing upon the technique of column chromatography and applying existing column stationary phases, a variety of sorptive extraction techniques have been developed. Since the 1970's solid phase extraction (SPE) has become a widespread and effective sample preparation technique for extracting analytes from complex matrices. The technique ranges in complexity from hydrophobic or hydrophilic partitioning to ion exchange or affinity chromatography.

The term "solid-phase" or "sorbent extraction", frequently abbreviated to "SPE", simply implies a physical extraction process involving a liquid and a solid phase. In practice, it has come to mean the use of commercial pre-packed columns containing stationary phases related to those used widely in high-performance liquid chromatography (HPLC). Sorbents for SPE are packed in different designs: solid disks, cartridges, and a variety of syringe barrels, where selective sorption and elution of desired analytes remains the heart of each design. The samples are loaded onto the SPE device where the analytes of interest bind noncovalently to the sorbent and the impurities flush through. The SPE membrane containing analytes bound to the sorbent can be washed to remove contaminants and the analytes are subsequently eluted using appropriate solvents.

SPE has many advantages over traditional extraction techniques. It uses smaller amounts of solvent, is relatively straightforward to use and is also cost-effective. Other attractive features of SPE include the ability to automate and adapt for on site and field analysis. There has also been the trend of moving SPE beyond the traditional cartridge format and placing it online with LC. This hyphenated technique has been used for sample preparation to clean up pollutants from biological samples prior to instrumental analysis [6,7].

The most popular type of bonded phase SPE is octadecyl-bonded silica, although SPE procedure employing silicas with other alkyl- or aryl- groups (C_2, C_8, diol, phenyl, cyanopropyl) have been reported. Many of the initial publications on bonded phases focused on hydrocarbons [7-11] and later on to pesticides [12,13] phthalates [14] and phenols and chlorophenols [15,16]. The wide spectrum of compounds that could be

extracted using bonded silica initiated numerous comparative studies on standard LLE methods.

Recent adaptations of SPE devices have evolved from using individual columns and cartridges to high throughput 96-well plates [17]. These high performance plates provide a means of high throughput solid phase extraction by processing 96 samples in parallel that can be extracted in approximately one hour or less. Each well of the plate contains the standard particle-loaded membrane for efficient sample extraction followed by elution in as little as 100 μL with mobile phase or mobile phase compatible solutions that can be injected directly onto a chromatographic system.

SPE cartridges, disks and microwell plates have reduced many limitations of classical extraction techniques. Although SPE uses less solvent, it is still a time consuming, multi-step procedure often requiring a concentration step, which may result in loss of volatile analytes. SPE also have some limitations such as matrix interferences for certain analytes, low recoveries, and clogging of the sorbent pores. The materials used for the construction of SPE cartridges inevitably contain plasticizers, such as phthalates, which are strongly UV-absorbing and have chromatographic properties that can lead to interference with further analyses. Although SPE is faster than conventional extraction techniques, the method still requires several steps including activation, conditioning, sampling, washing, drying and elution. Additional concentration of analytes may also be necessary for extracts where high sensitivity is desired. Although SPE manufacturers have improved the performance of disks and cartridges, problems such as high background and batch-to-batch variations still exist.

Solid phase micro extraction (SPME) is a sorption extraction technique that is very effective, and becoming increasingly popular, without having any of the limitations of the classical sample preparation techniques. This method is a solvent free extraction technique that presents many of the characteristics necessary to redress the limitations inherent in classical sample preparations previously discussed. SPME was first developed by Pawliszyn and his group in 1989 at the University of Waterloo (Ontario, Canada). They published the initial concepts of SPME in 1989 and following rapid development, resulted in the first SPME device in 1990 [18,19]. The invention of SPME appears to be a logical development based on open-tubular capillary columns used in GC. These capillaries had their break-through in the 1980's and the creation of SPME may have derived from this design. In 1993, Supelco (now Sigma-Aldrich) commercialized the SPME device, based on a reusable micro syringe, with polydimethylsiloxane and polyacrylate coated fibers.

SPME , which is described in detail in Chapter 1, integrates sampling, extraction, concentration and sample introduction into a single solvent-free step. Analytes in the sample are extracted and concentrated directly in the extraction fiber and a cleanup step is not necessary because of the selective nature of coatings [20,21]. SPME was introduced to analyze relatively volatile compounds, but the range of use has been extended to the analysis of a great variety of matrices including solid, liquid and gas [22-26]. The method has been routinely used in combination with GC and successfully applied to a wide variety of volatile and semi-volatile organic compounds [27,30].

SPME, which has proven to be as useful as conventional extraction techniques such as LLE, Soxhlet extraction, SFE and SPE is solvent-free, fast, simple, easily

automated and relatively inexpensive. The technique has two steps. In the first step, the fiber is exposed to the sample of headspace where analytes partition from the sample matrix to the coating. In the second step, analytes from the fiber is transferred to the instrument where desorption of the analytes occur. Because of its design and solvent-free nature, SPME can be easily interfaced to various types of instruments. Also because of its non-volatile extracting phase, only extracted analytes are introduced into the instrument. SPME is easily coupled to gas chromatography (GC) and with some modifications, to liquid chromatography (HPLC). Desorption is normally attained by placing the fiber into a hot injector in a GC instrument [31,32] or in a liquid desorption interface for HPLC analysis [33,34]. SPME has also been used in tandem with super critical fluid chromatography [35,36], capillary electrophoresis [37], and micellar electrokinetic chromatography [38,39].

Numerous applications of SPME has been reported in several review articles and books published mainly by Pawliszyn and co-workers [40-47]. The extensive applications cover various scientific fields, such as pharmaceutical, environmental, food, agricultural, forensic and medical sciences. One of the main applications of SPME is the determination of environmental contaminants such as pesticides, PCBs, PAH and other chemical compounds. In 1996, Jinno et al determined common pesticides in river water using SPME-LC coupled by the specially designed interface [48]. In 1998, Pawliszyn et al introduced SPME-LC with mass spectrometric detection for analytes in sludge and sediments [49]. The extraction of sixteen PAHs in sediment samples was demonstrated by Cam et al using SPME [50].

SPME has also widely been used for the extraction of analytes from pharmaceutical and biological matrices. In 1997, Volker et al determined eleven corticosteroids and two steroid conjugates in urine samples using an SPME-LC-MS system [51]. The following year Kosher et al reported the analysis of lidocaine in urine by SPME-LC [52]. In 2002, Walles et al investigated the automation of in-tube SPME with the quantitative LC-MS of drug metabolites [53]. Kataoka et al reviewed the applications of SPME for food analysis by GC and LC [54]. Snow et al showed that using on-fiber derivatization, the applicability of SPME is extended to include non- and semi-volatile analytes. The extraction, derivatization and analysis of estrogens and anabolic steroids were successfully developed [55,56].

The properties of the coating material are crucial for the partition process in SPME. The coating material that was used on the first commercial fused silica fiber was polydimethysiloxane. Since then several different kinds of coating materials have been introduced to enhance sensitivity and selectivity. Because Carboxen has a high capacity for volatile organic compounds, the first attempt to combine Carboxen with SPME was reported by Chai and Pawliszyn in 1995 [57]. Eventually, a new 75um Carboxen-PDMS mixed coating fiber was designed to increase the efficiency of SPME using Carboxen, and was commercialized in 1997 by Supelco [58]. There are currently two distinct types of SPME fiber coatings currently available from Supelco. The liquid coatings that extract via absorption are the homogeneous pure polymer coatings polydimethylsiloxane (PDMS) and polyacrylate (PA). The second group of coatings including polydimethysiloxane/divinylbenzene (PDMS/DVB), carbowax (CW), carboxen/ polydimethylsiloxane (CAR/PDMS), divinlybenzene/carboxen/polydimethylsiloxane

(DVB/CAR/PDMS) and carbowax/templated resin (CW/TPR) are mixed coatings, in which the extracting phase is a porous solid.

Limitations of SPME includes the fragility of fibers, which can be easily broken or damaged during agitation or injection and contamination of the coating after exposure to dirty samples. High molecular mass compounds can adsorb irreversibly to the fiber, thus changing the properties of the coating and making it unusable. The problems associated with complex samples are one of the reasons that poor reproducibility and linearity are sometimes observed when extracting analytes from dirty matrices. Despite the few limitations in this preparation technique, the availability of new fiber coatings that extend the range of application to other group of compounds, as well as more advanced features and application of field devices, demonstrates that SPME is a good replacement to the classical sample preparation techniques.

Although SPME is a simple and rapid technique for sample preparation, the applicability of SPME is occasionally limited by the small amount of coating material that is present on the fiber. The amount of PDMS used in SPME is typically in the order of 0.5 μL or less, thereby limiting the enrichment power of the technique and resulting in low extraction efficiencies. In 1999, Baltussen and co-workers introduced a novel technique called stir bar sorptive extraction (SBSE) that uses a stir bar coated with polydimethylsiloxane [59]. The method is based on the same principles as SPME where magnetic stir bars are incorporated into a glass jacket and the outer surface is coated with a layer of PDMS. In this approach, 50-300 µl PDMS coatings are used resulting in an increased sensitivity factor of 100 to 1000 compared to SPME.

PDMS coated stir bars are commercially available form Gerstel in two sizes: 10mm L x 3.2mm o.d. and 40mm L x 3.2mm o.d. The stir bar is introduced into an aqueous sample and extraction takes place during stirring. After extraction, the stir bar is removed from the sample, placed in a glass thermal desorption tube, placed in a thermal desorption unit and analytes are thermally desorbed. Alternatively, liquid desorption can be used to transfer analytes to the instrument.

Stir bar sorptive extraction has been applied successfully in environmental, food and biological samples. In 2000, Tredoux et al reported the determination of benzoic acid in lemon flavored beverages by SBSE-GC/MS [60]. The group reported a method linear range of three orders of magnitude and a repeatability of less than 5%. In the same year the extraction. and analysis of flavor in orange juice and pesticides in wine were described [61]. Many other successful application of SBSE has been reported in the determination of wines, fruits, dairy and beverages [62-65].

The success of SBSE in environmental sample extraction is due to the fact that the PDMS sorbent is ideal for volatile organic compounds and semi-volatile compounds. Field analysis is also very practical using SBSE because of its solvent-less characteristics and small size. Successful applications include volatile aromatic, halogenated solvents, polyaromatic hydrocarbons (PAH), polychlorinated biphenyl (PCB), pesticides, odor compounds and organotin compounds [66-69]. SBSE has also been applied to test for contaminants in drinking water, where analytes can be extracted with very high sensitivity, reproducibility and accuracy [70,71].

Since the development of SBSE, the technique has also been applied to the determination of organic compounds in biological fluids. Various substances have been

extracted from urine, serum and plasma. Some of these substances include phenols, steroids, fatty acids and drugs of abuse [72-75]. Other applications of SBSE reported include the determination of polynuclear aromatic hydrocarbons in urine and the PCBs in sperm [76,77].

In most of the studies done to date on SBSE, thermal desorption (TD) is generally used for the desorption of the analyte from the PDMS stir bar and analysis is carried out by gas chromatography–mass spectrometry (GC–MS). In addition, liquid desorption (LD) by an organic solvent has been done, and the organic solvent is analyzed by high-performance liquid chromatography (HPLC). SBSE can be a rapid and sensitive alternative to commonly applied sample preparation techniques such as SPE, LLE, SFE and even SPME. Future development of SBSE will include the application of it to gaseous samples, efficient interfaces to instrumentation other than GC and the development of stir bars coated with different sorbents.

The need for miniaturization, speed, solvent-free and more efficient instrumentation has been a topic of growing interest over the past decade. During this time, significant technical effort has been invested in the development of the primary separation component of the various analytical systems such as microcolumn LC and fast GC. The need for enhancement of sensitivity is also propelling the development of sophisticated instrumentation with a wide range of highly sensitive detectors and numerous other improvements to meet the demands of the pharmaceutical, chemical, environmental industry and also homeland security.

Some believe that today's vision of "Lab-on-a-Chip" will become a commonplace in laboratories within the next several years, replacing many classical instrumentation

techniques. Miniaturization includes substantial scaling down of sample sizes to nanoliters instead of milliliters and analytical devices such as nanoscale HPLC columns and sub microliter volume spectroscopic detectors. The current literature reveals a steady increase in publications exploring the potential benefits of miniaturized, solvent-free devices as well as strategies, which can benefit from microfluidics, and related issues.

Miniaturization, along with real-time, solvent–free and sensitive analytical methodologies of sample preparation and instrumentation strongly supports the needs of field analytical chemistry (FAC), which is a rapidly growing area of chemical analysis in which the analytical measurement is completely accomplished at the site where the analyte is located. In the light of the 21st century worldwide threat of terrorism, real time data analysis has become even more critical with regards to public safety and the testing of explosives and chemical warfare reagents. FAC not only saves time, but also reduces the cost of analyses. Other real-time decisions such as environmental assessments, forensic results, production line analysis can significantly reduce the cost of an operation.

The instrument that is poised to become the premier method for fast, real time and on site analysis is ion mobility spectrometry (IMS). Applications of ion mobility spectrometry to field analytical chemistry are rapidly increasing due to the many valuable inherent characteristics of the technique, which includes portability, selectivity, high sensitivity and the elimination of solvent mobile phases and carrier gases. IMS is founded on the discovery that ions can be created at ambient pressure from radioactive materials and that these gas-phase ions can be characterized rapidly for mobility in a weak electric field [78].

The development if IMS in the early 1970's [79,80] followed an unconventional and irregular pattern whereby initial enthusiasm about the simplicity of the instrumentation and extremely low detection limits for IMS was followed by disappointment and rejection of the method. The downturn occurred because gas phase ion chemistry was poorly understood and drift tube design and data collection from that era restricted the study of fast ion molecular reactions [81].

During the past twenty years significant evolution occurred in both the instrumentation and the understanding of the underlying theory of response of IMS. While some discoveries have revealed that many challenges exist in terms of influencing gas phase ions at atmospheric pressure, other discoveries have revamped the opinions concerning the scope and capability of IMS in chemical analysis.

In the early twentieth century, Langevin made significant contributions in both the experimental and theoretical aspects of gas-phase ions in air at atmospheric pressure [82,83]. Models for ion mobilities were introduced demonstrating that ionized air was a mixture of several chemical species. Interest in the technique reappeared in the 1920's and 1930's with some modifications using mass spectrometers under a vacuum. Following World War II, interest spiked in gas-phase ionization and the effects of nuclear blasts on the ionization of air. From the 1950's to 1970's additional studies highlighted a growing interest in tropospheric and stratospheric chemistry and prompted a few investigations of ionic mobilities in air at atmospheric pressure.

A significant discovery in the 1970's by Lovelock that revolutionized the creation of the modern IMS was the observation that simple ionization devices respond to extremely low vapor concentrations of atmospheric pollutants [84]. Correlation between

mass spectrometry and IMS and the combination of GC to IMS and MS were also described during this period [85,86]. At the same time, Karasek conducted intense investigations and evaluation of the response of IMS to organic compounds with regards to elementary response characteristics, comparison to existing technologies, and the speculation about the significance of IMS as an analytical instrument [87-91].

By 1975, a general dissatisfaction with IMS appeared when users, seeking small simple alternatives to mass spectrometers and gas chromatography found the response to IMS puzzling and unsatisfactory. Over the next several years, interest in the technique declined dramatically as the technology was viewed as too complicated for practical use. The fact that the method had limited linear range, prolonged memory effects and serious matrix interference did not help the popularity, and development of the technique.

It was not until the 1980's that military establishments found IMS fundamentally attractive due to low detection limits, acceptable selectivity, and the potential for field determinations. In the 1980's and early 1990's IMS was connected to military establishments within the US and Europe. Several detectors were tested under actual field conditions for the detection of nerve gas and blister agents. Many of these ionization detectors became recognized as potentially valuable, but aspects of their performance were still somewhat limited. During this period, several universities and government laboratories and small companies began showing interest in the technology and initiated research programs to improve the technology and better understand the theory behind gas-phase ionization.

The advantages of IMS in terms of size, sensitivity power and weight have spurred the evaluation of the technique in the 1990's for field analysis, environmental,

industrial and medical testing. Over the past fifteen years improvements and advancements in IMS have been reported along with engineering innovations, data analysis improvements, and a wide range of applications. The two most popular IMS instruments commercially available are the Chemical Agents Monitor (CAM) from Graseby Dynamics and the Ionscan from Smiths Detection. Both of these instruments are based on traditional time-of-flight drift tubes and a linear voltage field with air flow along with a Faraday cup detector.

Photoionization and corona discharge ionization were investigated as alternative ionization schemes for IMS [92,93]. Modifications of the typical IMS designs have also prompted the development of a new high-field drift tube called Field Asymmetric IMS (FAIMS). This design is more ideal for coupling to a mass spectrometer and offers enhanced sensitivity [94]. Miniaturization of IMS has been impressive in the development of the hand-held Chemical Agents Monitor (CAM) unit for rugged field analysis. All utilities, data analysis, drift tube controls, and results display are included in the hand-held unit. The CAM is robust and it can operate at extreme temperatures and climatic conditions without the failure that might be expected for analytical instruments. The CAM was used extensively for chemical warfare monitoring during Desert Storm in 1992 and also resulted in the creation of the smaller mini-IMS [95]. The mini-IMS is palm-size with several enhanced features such as non-radioactive ionization sources and improved drift gas flow.

In 1990, Eiceman et al described the first delineation of complex spectra for mixtures using the spectra from individual components to screen solid pharmaceuticals using IMS. The vapors were characterized by IMS/MS to evaluate the air based

atmospheric pressure chemical ionization of components in analgesic medicines [96]. Later that year, there were several reports of using IMS as a detector after liquid chromatography, capillary gas chromatography and supercritical fluid chromatography [97-100]. In 1992, Fytche and co-workers investigated the spectral properties, interferences and detection limits of cocaine, heroin, amphetamine and LSD using IMS [101].

The role of IMS as an explosives detector was examined in 1993, where residues were collected on a membrane filter and subsequently analyzed. Experimental results showed that the limit of detection for some explosives to be approximately 200 pg [102]. The following year IMS was used to study the ionization and fragmentation pathways of alkanes and alkenes. The mobility spectra for alkanes showed sharp and symmetrical profiles while spectra for alkenes suggested fragmentation. Ion identifications were made using mass spectrometry, and ionization pathways were supported using deuterated analogs of alkanes and alkenes [103]. The detection of nicotine in air was also demonstrated by Keenan et al using IMS at the nanogram per liter levels [104].

The role of IMS for industrial applications was reported for the field screening of volatile organochlorine compounds in gases, soil, air and in organic solvents [105]. The same year, a novel ammonia analyzer based in IMS was described. The online system was capable of monitoring changes in aqueous ammonia concentrations over a period of twenty-four hours. Studies were undertaken to establish the limit of detection of the unit and evaluate the effect of ammonium ions under simulated process monitoring [106]. Another application soon to be explored was the utilization of membrane inlet ion mobility spectrometry for online measurement of beer in yeast fermentation. Good

agreement between the ethanol concentration by membrane inlet ion mobility spectrometry and the values declared on the beer bottle labels were shown [107].

The utilization of IMS to screen methamphetamine in hair was reported by Miki in 1997. The hair samples were digested prior to IMS analysis and the results obtained were in good agreement with their GC/MS determinations [108]. The following year another method using trihexylamine as an internal standard was proposed for a rapid procedure to screen human hair for designer drugs [109]. Another novel application of IMS was reported using a gas phase electrophoretic technique to differentiate between fresh and frozen/thawed meats and detect microorganisms in foods. The effects of refrigerated storage (spoilage) and different freezing temperatures were studied [110].

The strong interest in using ion mobility spectrometry for explosives and chemical warfare agents, spurred by the increased threat of terrorism, continued into the 21st century. In 2000, Asbury et al reported the use of an electrospray ionization technique for the analysis of common explosives from aqueous solutions. A mixture of TNT, RDX and HMX was used to demonstrate the high separation, linearity and sensitivity of the IMS system [111]. The same research group later separated and identified some chemical warfare degradation products using IMS with mass selective detection. Four degradation products from the G-Type nerve agent were separated in less than 15 ms and the method was applied to spiked river water [112]. The following year Buxton and Harrington reported the use of a new data analysis technique called simple-to-use interactive self-modeling mixture analysis (SIMPLISMA) to resolve spectral features of explosives with respect to temperature [113]. They showed that this technique

provides additional information on IMS detection features that would normally be missed by visual examination of IMS spectra.

Since 2002, there have been numerous developments in IMS technology and the technique has also been used in tandem with a wide array of mass selective detectors, modified inlets and various ionization schemes. Although the majority of the recent efforts to enhance the analysis of explosives, narcotics, illicit drugs, and trace chemical analysis, the applications of the technique has also broadened extensively to include pharmaceuticals, biological and microbiological analysis [114-116].

Only a decade ago IMS was viewed as an out-dated technology within the ion-molecule chemistry and vapor sensing community. The resurgence of IMS instrumentation and technique is related to its excellent detection limits and to practical considerations such as size, weight, and power advantages, when compared with well-established technologies such as mass spectrometry (MS) or gas chromatography/mass spectrometry.

Unfortunately, IMS analyzers are insufficient to ionize and separate all ions in a complex sample. The absence of selectivity of ionization via APCI reactions, mobility spectra can be impossible to interpretable because of peak overlap. Also charge exchange reactions and competitive ionization may result in failures of one or more analytes to ionize. This limitation has restricted the technique to relatively clean sample matrices consisting of a limited number of analytes.

IMS has become an important tool in the battle against terrorism. The technique is used extensively for explosives and chemical warfare agent detection and identification. The high sensitivity, ruggedness, and field deployable nature of the

instrument have driven the use of IMS for these applications. Although well suited for the task, IMS still has a number of issues to be resolved. A better understanding of the gas phase ion chemistry, which defines IMS response needs to be developed further. Although IMS will remain the instrument of choice for many field analysis detection applications in the near term, questions surrounding its effectiveness need to be addressed in detail before IMS can be considered as a long-term detection solution. In some applications, sampling has become the weak point of the detection chain. Coupling novel sorption extraction methodologies, such as SPME and SBSE to IMS could be one of the solutions to overcome the limitations of the technique.

CHAPTER

1

SOLID PHASE MICROEXTRACTION

1.1 INTRODUCTION

Solid phase microextraction integrates sampling, extraction, concentration and sample introduction into a single solvent-free step. Analytes in the sample are extracted and concentrated directly in the extraction fiber. Not only does the method save time and disposal costs, but it can also improve detection limits. SPME has been routinely used with gas chromatography (GC) and GC/mass spectrometry (GC/MS). It can also be used for direct coupling with high performance liquid chromatography (HPLC) in order to analyze weakly volatile or thermally labile compounds not amendable to GC. The technique has been successfully applied for a wide variety of volatile and semivolatile compounds in environmental, biological and food samples.

SPME is based on a modified syringe, which contains a stainless steel microtube within its syringe needle. The microtube has a 1 cm fused silica fiber tip which is coated with an organic polymer. The syringe is designed to move the fiber in and out of the needle, which allows exposure of the fiber during extraction and desorption and its protection during transfers and storage. Few modifications of the original design have been reported and several different configurations of SPME devices for field samplers were evaluated for the analysis of volatile compounds [117].

Figure 1.1 shows the schematic of a commercially available SPME device (Supelco, Bellfonte, PA) that is designed to be used with a color-coded replaceable fused silica assembly. The device consists of a stainless steel barrel, a plunger, an adjustable depth gauge, and a stainless steel retaining nut.

Solid phase microextraction methods consist of two processes. The first step is the partitioning of analytes between the fiber coating and the sample matrix; the second step is the desorption of analytes from the fiber into the analytical instrument. In the first step, the coated fiber is immersed directly into the sample or the headspace of the sample, where the analytes partition and concentrate. After equilibrium has been reached, or after a defined time point, the fiber is withdrawn from the sample. In the desorption step, the fiber is exposed to the inlet of the instrument, most commonly a GC injection port or a modified HPLC solvent desorption chamber [118,119]. The analytes are desorbed either thermally in the hot injector port or extracted by a solvent mobile phase and subsequently analyzed by a conventional instrument.

There are three common modes of SPME extraction: direct extraction, headspace extraction and membrane protected extraction. In direct extraction mode, the fiber is placed directly into the sample and analytes are transferred from the matrix to the coating. Agitation, stirring or sonication can significantly decrease the equilibration time. For gaseous samples, convection is sufficient to facilitate short extraction times.

In the headspace mode, the analytes are transported to the fiber through the headspace. This approach is beneficial for sample types such as biological matrices of

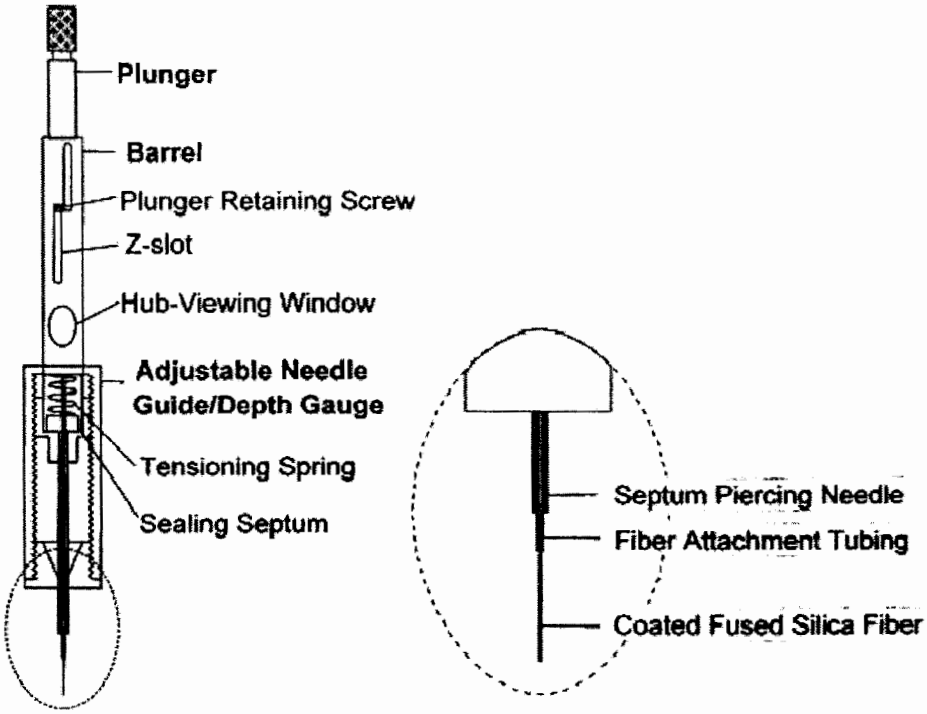


Figure. 1.1 Schematic of an SPME device showing the major components (Figure adapted from Reference 40).

humic matter, where the fiber is protected from damage by high molecular weight interferences. In headspace analysis, the fiber is also protected from harsh extraction conditions such as extreme pH and high salt concentrations. The issue of cross contamination is also minimized with headspace SPME because the fiber does not come in contact with the sample.

In membrane extraction mode, a membrane made from a special polymer can add a certain degree of protection to the fiber and also add selectivity to the SPME process. This membrane acts as a barrier to prevent detrimental matrix materials from destroying the fiber. This mode of extraction is advantageous for higher boiling point compounds that cannot be extracted by head space SPME. In membrane extraction the extraction time can be significantly longer than direct or headspace SPME, because analytes need to first penetrate the membrane before they can be absorbed or adsorbed to the fiber coating [120]. The three modes of extraction are shown schematically in Figure 1.2.

The detail process of sampling is shown in Figure 1.3. The sample is usually contained in a sealed vial. Extraction begins by piercing the septum with the SPME needle, and lowering the fiber into the sample, by depressing the plunger. Partitioning begins immediately between the sample matrix and the coating stationary phase. In headspace the fiber is exposed in the vapor phase above the gaseous, liquid, or solid sample. In direct immersion SPME the fiber is submerged in the liquid sample. After a suitable extraction time, the fiber is withdrawn into the needle, the needle is removed from the sealed vial and the fiber is then transferred to the instrument for desorption. The entire procedure can be performed manually or by an autosampler.

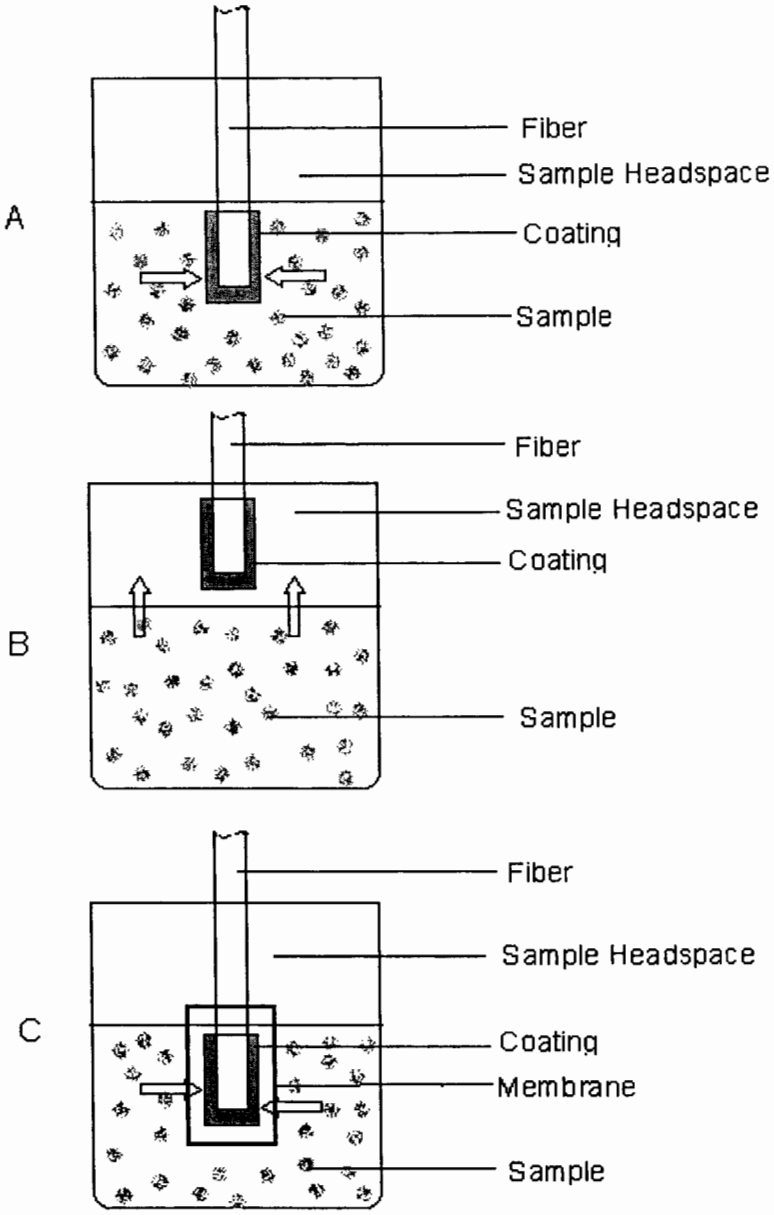


Figure. 1.2 The three modes of SPME operation: A. Direct Extraction, B. Headspace Extraction, C. Membrane Extraction

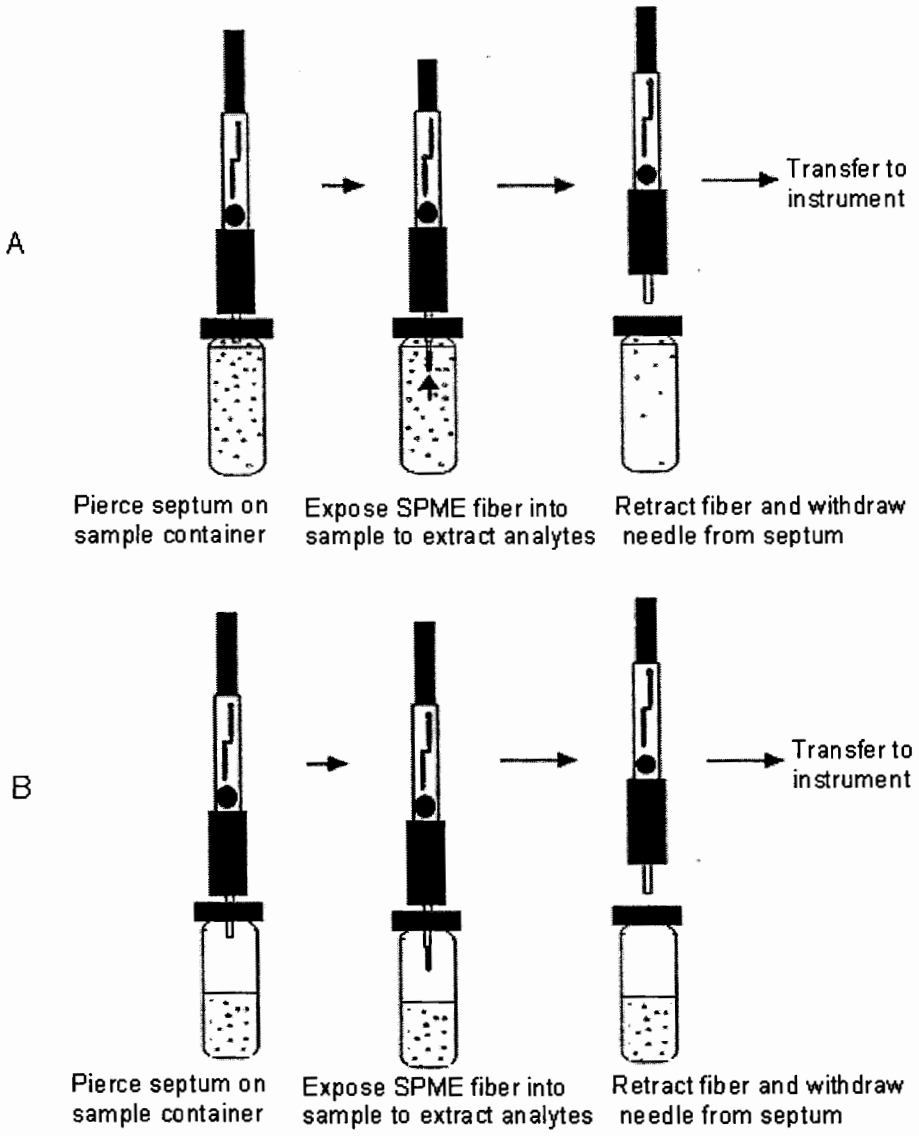


Figure 1.3 SPME procedures for A. direct extraction and B. headspace extraction

Since SPME is an equilibrium extraction technique, it has several inherent advantages over exhaustive extraction methods. In SPME it is not necessary to measure the volume of the extracted sample and therefore the fiber can be exposed directly to the sample source, such as a river or other location of interest. This characteristic of the method makes SPME ideal for field sampling. The equilibrium nature of the technique, along with its minute amount of coating, also allows the detection and quantitation of analytes in highly sensitive natural environments without perturbing the system. Equilibrium extraction techniques such as SPME also offer additional selectivity by utilizing the differences in extracting-phase/matrix distribution constants to isolate analytes. One disadvantage of equilibrium over exhaustive extraction is the requirement for calibration using surrogates, internal standards or standard addition.

The amount of analyte extracted by SPME at equilibrium is determined by the magnitude of the partition coefficient of the analyte between the two phases (matrix and coating). However, both the coating thickness and the partition coefficient determine the equilibrium time and the sensitivity of the method. Thick coatings will increase the sensitivity of the technique, but also negatively impact the method by extending the extraction time.

A general rule of thumb in SPME is to use the thinnest coating that will offer the required sensitivity to enhance the efficiency of the method. Although it is not necessary to calculate the partition coefficient when appropriate calibration techniques are used, it is often desirable to determine this parameter to aid in method development. The partition coefficient can be used to calculate the headspace volume, sample volume, and film

thickness required to achieve the necessary sensitivity. The equilibrium parameters in SPME can be described as: [121]

$$n = \frac{K_{fs} V_f V_s C_0}{K_{fs} V_f + V_s} \qquad \text{Eq. 1.1}$$

Where n is the amount extracted by the coating, K_{fs} is the fiber coating/sample distribution constant, V_f is the fiber coating volume, V_s is the sample volume and C_0 is the initial concentration of analyte in the sample. As see in Equation 1.1, which is the basis for quantitation, there is a direct relationship between sample concentration and the amount of analyte extracted.

The extraction of analytes in SPME can either be an absorption process, where analytes are absorbed into the coating polymer, or by an adsorption process where analytes are adsorbed on the surface of the coating. Absorption occurs in a liquid polymer coating phase and adsorption occurs when a solid phase is used. A schematic of the dynamics of absorption and adsorption is shown if Figure 1.4. While absorption is a non-competing process, adsorption is by definition a competitive process. In adsorption the molecules contend for the limited free space available on the surface of the solid. The selection of the coating used in SPME is based primarily on the polarity and volatility characteristics of the analyte.

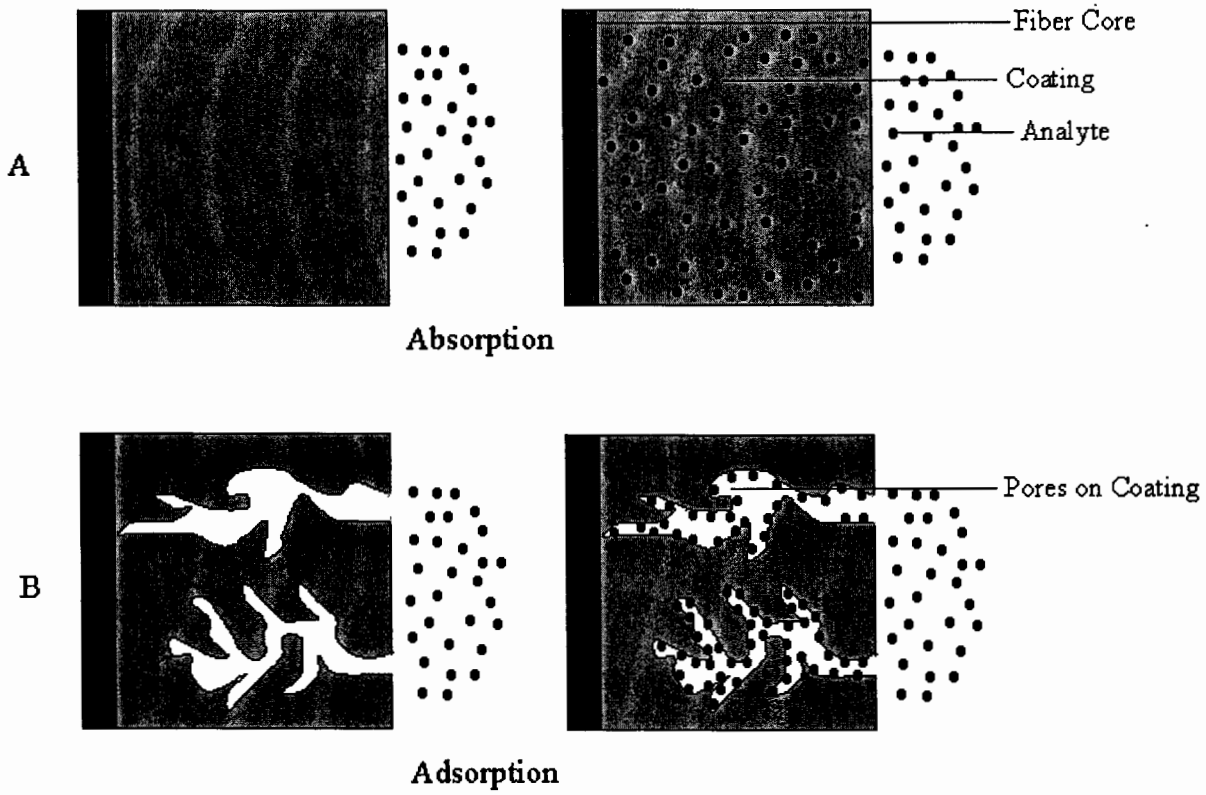


Figure 1.4 Mechanisms of A. Absorption, and B. Adsorption showing the dynamics of the sorption process on each fiber type.

The sensitivity and selectivity of SPME can be greatly enhanced by derivatization of analytes. This procedure can be performed before, during, or after the extraction process [122]. There are three different procedures that can be used: direct derivatization, derivatization on the SPME fiber, and derivatization in the injection port of the instrument. Compounds that are not amendable for SPME or a particular instrument, such as a gas chromatograph, can be derivatized to modify a functional group or change the chemical properties of the analytes to make the method feasible. Derivatization can be useful when very polar compounds have to be extracted. It can increase the volatility and reduce the polarity of analytes and therefore can improve extraction efficiency, selectivity, and detection. However, incorporation of a derivatization step can complicate the method, especially in field applications, and should only be used when absolutely necessary.

When SPME is used for quantitation, the calibration method can be critical for precision and accuracy. Usually the complexity and homogeneity of the sample and matrix will determine which technique to use. For relatively clean samples, external calibration standards or the distribution coefficient can be used. It is assumed that the distribution coefficients are very similar for the samples and the pure matrix. For complex samples isotopically labeled spikes or internal standard should be used. Standard additions calibration can also be used for complex samples.

1.2 THERMODYNAMICS

The theory of SPME has been widely presented by Pawliszyn and his group. The thermodynamic aspects of SPME have been thoroughly investigated and shown that the amount of analyte extracted by the coating is proportional to the analyte concentration in the sample, and is independent of fiber location. The thermodynamic driving force for molecular diffusion between phases is the decrease in free energy that analyte molecules experience in the favored phase. During extraction analytes migrate between the three phases; sample, headspace and fiber coating until equilibrium is reached.

The equilibrium of analytes in the three phases, where the mass remains constant in the system, can be described using the following equation [123]:

$$C_0V_s = C_f^{\infty}V_f + C_h^{\infty}V_h + C_s^{\infty}V_s \qquad \text{Eq. 1.2}$$

where C_0 is the initial concentration of the analyte in the matrix, C_f^{∞}, C_h^{∞}, and C_s^{∞} are the equilibrium concentrations of analyte in the coating, the headspace and the sample, respectively; V_f, V_h and V_s are the volumes of the coating, headspace and the matrix respectively. The fiber coating/gas distribution constant can be described as $K_{fh} = C_f^{\infty}/ C_h^{\infty}$, and the gas/sample distribution constant as $K_{hs} = C_h^{\infty}/ C_s^{\infty}$. The mass of analyte in the coating, $n = C_f^{\infty}V_f$ can be expressed as:

$$n = \frac{K_{fh}K_{hs}V_fC_0V_s}{K_{fh}K_{hs}V_f + K_{hs}V_h + V_s} \qquad \text{Eq. 1.3}$$

Since the fiber/headspace distribution constant, K_{fh}, can be estimated by the fiber/gas distribution constant K_{fg}, and the headspace/sample distribution constant by the gas/sample distribution constant, K_{gs}, the following equation can be deduced:

$$K_{fs} = K_{fh} = K_{fg}K_{gs} \qquad \text{Eq. 1.4}$$

Equation 1.3 can be rewritten as:

$$n = \frac{K_{fs}V_fC_0V_s}{K_{fs}V_f + K_{hs}V_h + V_s} \qquad \text{Eq.1.5}$$

If there is no headspace in the sample, the term $K_{hs}V_h$ in the denominator can be eliminated, giving rise to:

$$n = \frac{K_{fs}V_fV_sC_0}{K_{fs}V_f + V_s} \qquad \text{Eq. 1.6}$$

Equation 1.6 depicts the relationship of the analyte mass that is extracted by the fiber coating after the system has reached equilibrium. In most applications, K_{fs} is relatively small compared to the phase ratio of sample matrix to coating volume ($V_f << V_s$) resulting in a state where the capacity of the sample is much larger than the capacity of the fiber. Equation 1.6 can therefore be simplified to:

$$n = K_{fs} V_f C_0 \qquad \text{Eq.1.7}$$

The simplified Equation 1.7 demonstrates that in SPME it is not necessary to determine the sample volume as long as $K_{fs}V_f << V_s$.

Equation 1.7 assumes that the system exists as a single homogeneous phase and there is no headspace present. Since there is a proportional relationship between n and C_0, non-equilibrium conditions can also be used for certain applications where the experimental time needs to be shortened and high sensitivity is not desired [124]. As long as the sampling time is held constant, the extraction can be interrupted prior to equilibrium and the amount of analyte extracted by the fiber will correlate to the initial concentration of the sample.

As discussed in section 1.1, it may be desirable to determine the distribution constant to aid in method optimization. For direct extraction mode this parameter can be calculated from the following equation deduced from Equation 1.6:

$$K_{fs} = \frac{nV_s}{V_f(C_0V_s - n)} \qquad \text{Eq.1.8}$$

The distribution constant calculated from Equation 1.8 can also be estimated from physiochemical data and chromatographic parameters. Isothermal gas chromatography can be used to determine K_{fg} where the stationary phase of the column is identical to the polymer coating. This determination is possible because the retention mechanism of analytes in a GC column is similar to the partition of analytes in SPME [125]. The

distribution constant has also been correlated with the octanol/water distribution coefficient, K_{ow}, since this is a classical and general measure of the affinity of analytes to the organic phase.

Thermodynamic principles dictate that modifying certain extraction conditions can significantly affect the partition of analytes to the fiber coating. The amount of analyte sorbed on the SPME fiber and the resulting sensitivity are determined by the sorption kinetics and the distribution constants of the analytes between the sample and fiber surface. Extraction conditions that affect the distribution constants of analytes between the sample and fiber include temperature, salting, pH and organic solvent content modification.

Temperature plays an important role in the partitioning of analytes from the sample matrix to the polymer coating. The distribution constant changes are reflected by the following equation when both sample and the fiber change temperature from T_0 to T:

$$K_{fs} = K_0 \exp -\frac{\Delta H}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right) \qquad \text{Eq. 1.9}$$

where K_0 is the distribution constant when the sample and fiber are at temperature T_0, ΔH is the molar change in enthalpy of analyte when it migrates from sample to fiber coating, and R is the gas constant. In SPME the enthalpy change is considered constant over the typical operating ranges. When the K_{fs} value for an analyte is greater than 1, the analyte has a lower potential energy in the fiber than in the sample, so the analyte partitioning

into the coating is an exothermic process. The thermodynamic theory dictates that raising the temperature of the sample and fiber will decrease K_{fs}. Increasing the temperature of a sample prompts an increase in the release of analytes from the matrix and also favor the extraction of semivolatile compounds. However, because the distribution constant decreases at elevated temperatures, the amount of analyte transferred to the fiber is correspondingly less and there is a loss of sensitivity for the method. The distribution constant can be significantly increased when the sample is heated and the fiber is cooled simultaneously. This benefit can be accomplished by using an internally cooled fiber to create a temperature gap. Although this device is not yet commercially available, it would greatly enhance the sensitivity and speed of extraction.

1.3 KINETICS

Kinetics of the transfer of analytes form the sample matrix to the fiber coating governs the speed of extraction. The theory is very useful for optimizing the method parameters to achieve maximum sensitivity in the shortest possible time. Fick's second law of diffusion governs the theory of mass transport in a dynamic SPME system, where factors such as thermal expansion, swelling and analyte/analyte interactions are assumed to be negligible.

The model most commonly used to explain SPME kinetics is the perfect agitation model, where the sample is perfectly agitated. All analytes in the sample are assumed to

have access to the fiber coating where the equilibration time, t_e, defined as the time required to extract 95% of the equilibrium amount is:

$$t_e = t_{95\%} = \frac{2(b-a)^2}{D_f} \qquad \text{Eq. 1.10}$$

where, a is the fiber coating inner radius, b is the fiber coating outer radius, D_f is the analyte diffusion coefficient in the fiber coating. Figure 1.5 shows a graphic representation of the system. As soon as the fiber is placed in the sample matrix, there is a rapid transfer of analytes to the fiber surface. The rate of increase then slows and eventually reached equilibrium.

Equation 1.10 can be used to estimate the fastest equilibration time by substituting the diffusion coefficient of an analyte in the coating and the fiber coating thickness. Experiments have been done where the measured equilibration times have been close to the predicted equilibration time. These results were obtained in gaseous samples, where the diffusion coefficient is very high or when sonication was used to enhance mass transfer in liquids.

Under practical conditions, there is always a thin layer of stagnant analyte solution around the fiber called the Prandtl boundary layer. Such layers are common for solutions in contact with surfaces. The thickness of this layer is determined by the agitation rates and the viscosity of the matrix. When the stirring rate is high this layer get smaller. The boundary layer model configuration is shown in Figure 1.6.

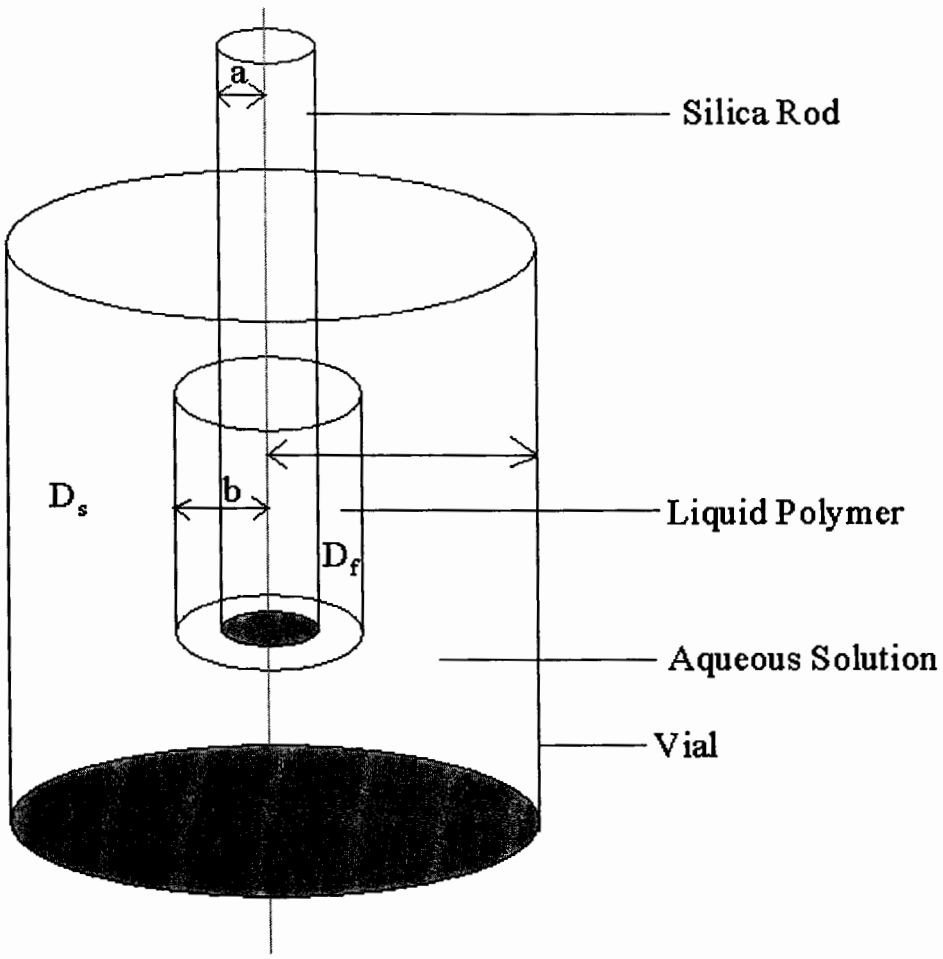


Figure 1.5 Schematic of the SPME and sample system configuration showing the parameters, a, the fiber coating inner radius, b, the fiber coating outer radius, D_f, the analyte diffusion coefficient in the fiber coating and Ds, the analyte diffusion coefficient in the sample

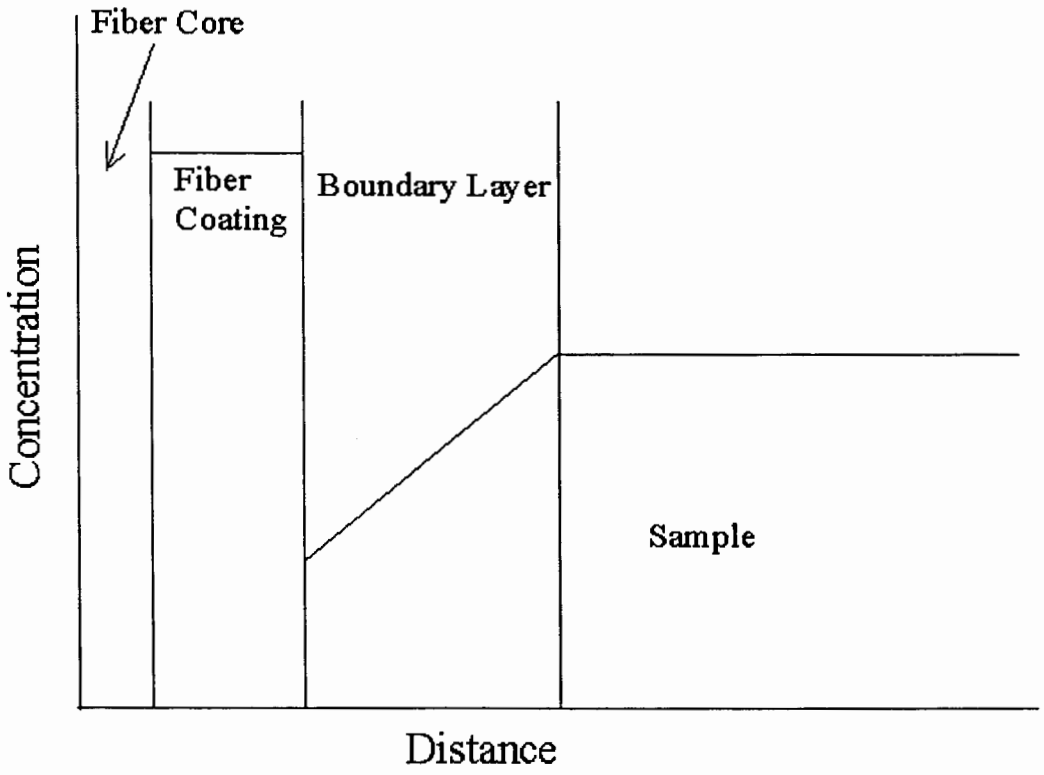


Figure 1.6 Graphic representation of the boundary layer configurations that analyte transverse. The boundary layer determines extraction rates.

For practical applications of SPME, the equilibration time can be estimated form the following equation:

$$t_e = t_{95\%} = 3\frac{\delta K_{fs}(b-a)^2}{D_s} \qquad \text{Eq. 1.11}$$

Where K_{fs} is the analyte distribution coefficient between the sample and the fiber, and D_s is the analyte diffusion coefficient in the sample. Equation 1.11 can estimate the equilibration times when the extraction rate is governed by the diffusion in the boundary layer. The extraction times predicted from Equation 1.10 is shorter than the time calculated from Equation 1.11.

The equilibration time is defined as the time after which the amount of analyte extracted remains constant within reasonable experimental error at infinite extraction time. As shown above, kinetics predict that the equilibration time for well agitated samples is proportional to the thickness of the polymer coating. If agitation is not used or is at its optimum, the bottleneck step in SPME rate of extraction is the thickness of the boundary layer.

In headspace analysis, equilibration times are often short and limited only by the rate of diffusion of analytes in the coating. When the liquid and gaseous phases are at equilibrium prior to the beginning of extraction, most of the analytes are present in the headspace. As a result the extraction times are frequently short even when no agitation is used. When the temperature is high enough to transfer analytes from the matrix to the headspace, extraction occurs only from the headspace and the equilibrium time is very short and it is independent on agitation.

The efficiency of the agitation technique determines the equilibration time in aqueous samples. The techniques used for agitation are magnetic stirring, needle vibration, vial vortexing, and sonication. Magnetic stirring is the most convenient, practical and common technique used for SPME because it can be used in different SPME sampling modes and it is readily available in most laboratories. Disadvantages of magnetic stirring include difficulties in maintaining a constant speed, and the potential for carryover. The equilibrium times progressively decrease as the rpm increase [126]. The needle vibration technique uses a motor to generate a vibrating motion of the fiber and vial. This technique is used by the Varian commercial autosampler and provides good agitation, resulting in equilibration times similar to magnetic stirring. In the vortexing technique, the vial is moved rapidly in a circular motion. The most effective agitation technique for SPME is direct sonication, which provides very short extraction times. However, this approach may heat the sample and in some case destroy analytes.

In addition to agitation, additional parameters such as temperature, distribution constants and fiber coating thickness have an effect on SPME equilibration times. An increase in extraction temperature results in an increased diffusion coefficient and decreased distribution constant, both leading to a shorter equilibration time. Therefore for most applications, temperature can be optimized to attain the desired sensitivity in the shortest possible time. In headspace analysis, a higher temperature results in a higher concentration of analytes in the gas phase, which facilitates faster extraction. As seen in Equation 1.10, the thickness of the fiber coating could have a significant impact on the time of extraction. Coating thickness changes not only the amount of analyte extracted,

but also the equilibration time. To improve method efficiency it is advisable to use the thinnest coating that will achieve the required sensitivity.

The distribution constant of an analyte between the matrix and fiber coating also affects the equilibration time of SPME. As the distribution constant increases, the amount of analyte extracted increases, but the equilibration times become longer as well. Analytes that have high affinities for the coating will reach equilibrium later than analytes with low affinities toward the coating.

1.4 COATINGS

Coating selection is a crucial step in any SPME method optimization. The chemical nature of the analyte determines the type of coating used. There are a few simple rules that should be applied in coating selection when a new SPME method is attempted. The polarity of the coating should match the polarity of the analyte according to the rule, "like dissolves like". The volatility characteristics should also be considered. Another coating selection factor is the ability of the polymer to withstand extreme chemical and physical conditions, such as pH, salts, and high temperatures if such conditions are employed in a method.

The number of groups forming hydrogen bonds is an important property of analytes. For example, the number of amino or hydroxyl groups mainly determine the hydrophilicity of an analyte, and therefore the affinity of the coating. Numerous types of polymers have been investigated for a range of applications. In addition the commercially

available polymer coatings, several customized coatings have been developed for specialized applications [127,128]. Examples of specialized applications include ion exchange coatings to isolate metal ions and proteins, liquid crystalline film coatings to extract planar molecules, pencil leads and Nafion coatings to extract polar analytes [129-133].

The uniform distribution and thickness deposition of the coating onto the fiber is critical during the manufacturing process of the SPME device. The most straightforward technique used to deposit coating onto a fiber is the dipping technique where the fiber is placed for a short time in a concentrated organic solvent solution of the material to be deposited. The solvent is then evaporated leaving the coated material on the fiber. Electrodepostion, which is an extension of the dipping technique, can also be used to selectively deposit polymer coatings on the surface of metallic rods. The disadvantage of this approach is the poor reproducibility of the film thickness deposited. In commercial devices, where the principles of optical fiber preparations are adapted, and where film thickness reproducibility is excellent, the preparation of films is carried out simultaneously during the drawing of the fused silica rod.

A compromise is necessary with regards to the coating thickness between sensitivity and speed of extraction. The choice of thickness selection in fiber coatings is limited to PDMS, where this is the only coating commercially available in different sizes. If maximum sensitivity is the greatest concern and extraction time is not a factor, then a 100 μm coating can be used. If short extraction time is desired over high sensitivity then a 7μm coating can be used. Currently the following coating types are commercially available:

a. Polydimethylsiloxane (PDMS)

b. Polyacrylate (PA)

c. Polydimethylsiloxane/Divinylbenzenen (PDMS/DVB)

d. Carbowax/Polydimethysiloxane (CW/PDMS)

e. Carboxen/Polydimethysiloxane(CAR/PDMS)

f. Carboxen/Polydimethysiloxane/Divinylbenzene (CAR/PDMS/DVB)

There are four absorbent (liquid) type fibers, which include the nonpolar PDMS in three different coating thickness (7 μm, 30μm and 100μm) and the polar PA fiber, which is available in one thickness (85μm). The adsorbent (solid) type fibers contain either DVB and/or Carboxen. Depending on the desired polarity, the DVB fibers are available suspended either on PDMS or Carbowax, a moderately polar phase. Carboxen is available suspended in PDMS. There is also an additional fiber available that contains a combination of DVB/PDMS layered over Carboxen PDMS.

The liquid absorbent type fibers works like a sponge and extracts by partitioning where the analytes migrate in and out of the coating. The ability of the coating to partition analytes depend on the thickness of the coating, the size and polarity of the analyte. In liquid phases, thicker film coatings have higher sample capacity and there is no competition between analytes.

In the solid and mixed bed coatings, which contain pores or high surface areas, adsorption occurs where the fibers physically interact with the analytes. The micro and meso pores are well suited for trapping and retaining analytes until they are displaced by applying energy or by a solvent. In adsorption SPME, there is reduced analyte capacity

and analytes with lower distribution constants can be displaced from the coating because they compete for a limited numbers of sites on the fiber.

The most popular coatings are the PDMS coatings. These liquid fibers are very rugged and can withstand high injector temperatures (up to 300°C). PDMS is a nonpolar phase which also extracts nonpolar analytes very well [134-138]. They can also be used to extract polar analytes after optimizing conditions such as temperature, pH, and salt concentrations. An additional advantage of using PDMS is the ability to determine the distribution constants of analytes in air from gas chromatography retention calculations, since this coating is also used as a column stationary phase [139-140]. Polyacrylate coatings, which have lower diffusion coefficients than PDMS, and therefore longer extraction times, are suitable for more polar analytes, such as phenols [141].

The mixed phase coatings, which exhibit complementary properties to the liquid coatings, have higher distribution constants compared to PDMS and are more suitable for volatile analytes. Changing the liquid phase from PDMS to Carbowax, or vice versa can alter the selectivity of the mixed beds. DVB coatings exhibit additional selectivity by excluding high molecular weight compounds, which cannot penetrate the small pores. The smaller linear range and the displacement effect discussed earlier are a few disadvantages of the mixed bed coatings.

1.5 OPTIMIZATION OF SPME EXTRACTION PARAMETERS

Extraction Mode. The matrix composition, analyte volatility and its affinity for the fiber determine whether a headspace or direct extraction technique is selected. For complex

samples that could damage the fiber, a headspace or membrane protection mode should be used. For clean samples any of the above techniques can be applied. However, headspace is applicable for more volatile analytes. It is also the preferred mode because equilibration times are usually shorter than direct extraction, resulting in faster analysis. The diffusion coefficients for gases are typically four orders of magnitude larger than the liquid phase, where the extraction time is determined largely by the diffusion in the vapor phase. The headspace mode is also more amendable to pH and salt modifications to improve extraction efficiency.

Fiber Selection. In SPME the chemical nature of the analyte determines the type of coating used. The like dissolves like rule should always be applied for a successful method, where the polarity of the analyte should match the polarity of the fiber. As discussed in Section 1.4, the PDMS coatings are usually attempted first in method optimization due to their robust characteristics. Although this coating is ideal for nonpolar compounds, it can also be applied for polar compounds with additional method modifications. The solid coatings, usually available in the mixed bed configuration, also offers addition options in terms of selectivity and sensitivity. When a broad range of analytes need to be extracted, method optimization may involve the use of multiple fibers. Since no single coating will work to the same extent for all analytes, a compromise in terms of sensitivity may be necessary to extract all analytes with one fiber.

The coating thickness is also an important factor to consider during method optimization. It affects the selectivity of analytes, extraction time, sample capacity, desorption time and the amount of potential carryover. Usually the thinnest fibers, which

achieve the desired sensitivity should be used. The thick coatings, such as the 100 μm PDMS fiber offer increased sensitivity, but require much longer equilibration time. A compromise is necessary with regards to coating thickness selection with respect to sensitivity and speed of extraction of a method. The desorption mechanism, whether thermal or solvent, is also important in the fiber selection process. The current SPME vendor, Supelco, offers general guidelines with the purchase of fibers to aid in method development.

Agitation. Agitation is normally used to achieve faster equilibration because it enhances the diffusion of analytes toward the fiber. The effectiveness of the agitation technique determines the speed of extraction in direct mode SPME. The techniques discussed in Section 1.3 are magnetic stirring, vortexing, fiber movement, flow through and sonication. Since magnetic stirring capabilities are available in most laboratories, it is the most common technique used for agitation. Digitally controlled stirrers are ideal since it is important that all extractions are performed at the same setting. Sonication, which is capable of producing almost theoretical extraction limits, has been proven to be the most efficient agitation technique in SPME. The Varian autosampler. conveniently automated to process a large number of samples, uses a shaking motion to agitate the sample vial and fiber. The speed of agitation can be controlled.

Extraction time. The amount of time necessary to reach equilibration in SPME is dependent on the mass transport conditions, nature of the analyte and several other extraction parameters such as temperature, pH etc. In most SPME optimization scheme, the analyte is allowed to reach equilibrium between the sample and the fiber coating. The extraction time profile is obtained by preparing samples and then extracting them for

progressively longer periods of time. Equilibrium is reached when no additional amount of analyte is transferred from the sample to the coating within reasonable experimental error. Non-equilibrium conditions can also be used in SPME when the equilibration time is excessively long and sensitivity of the technique is not of greatest concern. However, extraction time and agitation must be strictly controlled to ensure good reproducibility of the method.

A graph of the relationship between peak response and time is useful in method optimization. The equilibration time can be obtained when no further increase of peak response is observed with increased time of extraction. Depending on the slope of the extraction time profile, the amount of error can be significant if the equilibration time is incorrectly assigned. Use of internal standards can compensate for some of these errors.

The equilibration time is usually shorter for analytes with low distribution constants, since less analyte is transferred to the coating. When a sample is unstirred, the analytes will only reach the fiber by diffusion and this can be a relatively slow process for diffusion in liquids. Any kind of stirring will shorten the extraction time. In headspace analysis the equilibration time is usually shorter for volatile compounds compared to semi- and nonvolatile ones, owing to higher diffusion coefficients. In both direct and headspace SPME, temperature can have an important effect on the equilibration time.

Temperature. Increasing the temperature of the system in SPME increases the extraction rate, but at the same time decreases the distribution constant. A plot of analyte response against sample temperature facilitates method optimization and the selection of the correct temperature for the most efficient method. If the desired sensitivity is achieved at higher temperatures, then the elevated temperature should be used in the method to

facilitate faster equilibration time. In general, the highest possible temperature should be used. In headspace analysis, increasing the temperature of the liquid phase, facilitates the transfer of analytes into the headspace and helps to speed up the rate of extraction. An internally cooled fiber, which is not yet commercially available is an opting to improve the extraction speed without compromising the sensitivity of the method.

The enthalpic effects in SPME were described in Section 1.2. Since temperature affects both the distribution constant and the diffusion coefficient of analytes, care should be taken to avoid the inadvertent heating or cooling of the sample during extraction. Heat can be introduced during stirring, sonication or from other sources in filed analysis.

pH. The pH of the sample is critical in SPME when the analytes of interests are acids or bases. For these analytes changing the pH of the sample matrix determines whether the species exist as an associated or dissociated moiety and thereby offering method selectivity in terms of whether or not the analyte gets extracted. For example, in a non-polar coating, as the pH is lowered, more acidic analyte is present in neutral form making it more likely to partition into the coating, resulting in higher sensitivity. The highest sensitivity is achieved when the pH is two units lower than the pK_a value of the acid [142]. For basic analytes the pH must be two units larger than the pK_a value of the analyte. Modifications of pH should be done carefully during method optimization, since matrix changes can also affect the distribution constant of the analytes. In direct extraction the extent of pH modification is limited compared to headspace analysis, since the fiber can be damaged at the extreme conditions.

Salt Concentration. Increasing the salt concentration of the matrix often results in an increase in the amount of analyte extracted by the fiber coating. The opposite effect is

observed when ionic analytes are present in their dissociated forms. This effect is explained by the fact that the activity coefficient of the ionic species generally increases when the sample ionic strength increases. Therefore in SPME it is generally important to first convert the analytes into neutral forms. Partition coefficients are related to solubility, and the more analytes tend to remain in the aqueous phase, the lower the partition coefficient value. Since the aqueous solubilities of many organic compounds decrease in the present of excess salt, a simple way to change the solubility of analytes and hence increase the K_{fs}, is to add salt to the matrix.

Sample Volume. The distribution constant determines minimum sample volume that should be used in an SPME method. The minimum sample value can be calculated from the K_{fs} or it can be determined experimentally. This method optimization study is performed by extracting samples at successively larger volumes and plotting it against the analyte response. As seen in Equation 1.6, the sensitivity of the method is proportional to the amount of analyte extracted from the sample. As the sample volume increases, so does the amount of analyte extracted, until the volume of the sample becomes significantly larger than the product of the distribution constant and volume of the coating ($K_{fs}<<V_s$). At volumes larger than this cutoff value, the amount of analyte extracted is independent of sample volume. Using larger sample volumes is desirable because it not only improves sensitivity, but also the precision of the method since variations in sample volumes do not affect the results.

1.6 DESORPTION

The nature of the analytes, complexity of the samples, separation capability and sensitivity requirements determine the instrumentation used for detection and quantitation after SPME. Most SPME applications have been developed for gas chromatography, but more recently, commercial interfaces to HPLC have been designed. Coupling of SPME to instruments such as CE, SFC and MS has also been attempted. In GC, efficient desorption and rapid transfer of analytes from the injector to the column require high linear flow rates of the carrier gas around the coating. In HPLC the composition, temperature and flow rate of the mobile phase affect the efficiency of the desorption process.

During the desorption process, the extracted analytes transverse from the coating into the carrier gas or liquid. This process can therefore be described as the reverse of the extraction from a well-agitated sample when initially no analyte is present in the coating. A high flow rate is necessary to remove desorbed analytes from the layer of gas or liquid immediately adjacent to the fiber. This removal increases the concentration gradient and therefore facilitates the diffusion rate of analytes out of the coating. Initially, analytes are removed from the outermost layer of the coating. As desorption progresses, analytes are removed from deeper layers until the process is complete. Based on the theoretical kinetics of desorption, the time necessary to desorb analytes is dependent on the distribution constant, K_{fg}, of analytes in the coating.

Some of the parameters that determine the desorption time include analyte volatility, injector design, and temperature. As expected, more volatile analytes desorb

faster than less volatile analytes. Injector design is an important factor to consider for efficient SPME desorption. The fiber should be physically positioned in the injector such that it receives uniform temperature distribution and optimum flow rate of carrier gas or solvent. Since the linear flow rate of carrier media should be as high as possible for efficient desorption, the internal diameter of the desorber could be an important factor.

To facilitate sharp injection bands, the analytes desorbed should be removed as rapidly as possible from the fiber into the injection port of the instrument. The theoretical desorption times are very short since the diffusion coefficient of analytes increases rapidly and the gas/coating distribution coefficient decreases as the temperature increases. Also at high flow rates of the carrier gas, the injection temperature requirements can be lowered since analytes do not need to be fully partitioned into the gas phase to be quickly and exhaustively desorbed from the coating.

In any SPME desorption, carryover should be minimized or eliminated. Carryover studies can be done by re-injecting the fiber after the initial injection to determine the desorption efficiency. Adjustments of the desorption time and temperature, can be used to help quantify the desorption dynamics. Low temperatures may cause slow or incomplete desorption while excessively high temperature may destroy the coating.

1.7 APPLICATIONS

Although SPME devices have been used mainly in laboratory applications, there has been a growing trend towards remote field monitoring especially in areas such as

clinical, forensic, environmental and industrial hygiene. The fiber can be used to extract analytes from gases, the headspace of solids and liquids and direct immersion in a liquid matrix. Since the invention of SPME in 1990, the number of applications of the technique has grown exponentially, evidenced by the growing number of publications.

Most of the early applications of SPME focused on environmental applications for organic contaminants, pesticides, herbicides and other biologically active compounds in aqueous samples [143-148]. Headspace SPME has been widely used for the determination of volatile, semivolatile and organometallic compounds in sediments, soils and sludges. Some of the most common applications include, pesticides, fungicides, alkylbenzenes, aromatic amines, phenols, phthalates and polyaromatic hydrocarbons from environmental matrices. In addition to headspace SPME, direct SPME has also been used as an alternative to liquid-liquid extraction for these analyses. The majority of methods developed are based on GC/MS separation and detection, however, increasing use of SPME with HPLC is reported.

Food analysis is another area where SPME has been widely utilized for the evaluation of nutritional value, for the monitoring of freshness, additives and toxic contaminants. Frietas et al developed a method using headspace SPME to characterize coffee products [149]. The technique has also been combined with GC/MS to analyze aroma in Brazilian tropical fruits, essential oils from peppers, carcinogens in cigarettes, and fatty acids in cheese [150-154]. SPME has also been used to monitor the volatile flavor profile of fruits during the ripening and storage period and the emission of volatile organic compounds in mechanically wounded plants [155,156].

One of the most prevalent applications of SPME for food testing has been the analysis of pesticides, herbicides, fungicides and other agrochemical products. Headspace SPME has also been used to monitor the emission of hydrocarbons emanating from food product packaging materials. Ezquerro et al used Carboxen-PDMS fiber to quantitate 22 compounds formed by thermo oxidative degradation of polyethylene packing material [157]. The headspace extraction technique is also one of the most popular methods for the characterization of different alcoholic beverages, and the trace detection of analytes based on their volatile composition.

SPME has been utilized for a wide variety of drugs analysis in biological fluids and matrices. Although headspace extraction is ideal for complex matrices and numerous successful applications have been reported, direct immersion SPME is also possible for these samples. Headspace SPME works well for the analysis of biological samples as interferences from high molecular weight compounds is reduced, yielding cleaner extracts.

Much of the early SPME work on biological specimens focused on very volatile compounds, such as ethanol and solvents in urine. Since then however, a variety of drugs and environmental hazards such as amphetamines, antihistamines, corticosteroids, pesticides, organometallics, inorganic mercury and industrial chemicals can be detected and quantitated [158-162]. A popular application of SPME is the analysis of amphetamines and their metabolites in urine. Most of these analytes have also been extracted from blood, saliva and hair samples. Both headspace and direct extraction combined with HPLC/MS detection has been used for the analysis of these compounds.

The applications for environmental, food and drugs analysis presented above are just a small fraction of the immense array of analytes and matrix types that have been successfully developed for SPME. Future developments in SPME sample preparation are directed at developing smaller volumes of the extraction fiber and also in-tube extraction techniques. Miniaturization in sample preparation methods has been one of the most attractive and desirable techniques for the pretreatment of complex samples prior to instrumental analysis. This trend will continue as there is a continuous demand for fast, portable, solventless and sensitive methods of sample preparation.

CHAPTER 2

STIR BAR SORPTIVE EXTRACTION (SBSE)

2.1 INTRODUCTION

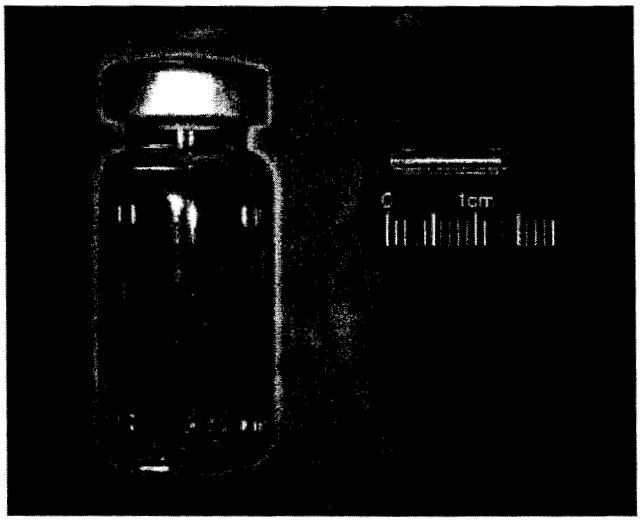
Stir-bar sorptive extraction is a relatively new solvent-less sample preparation method for the extraction and concentration of organic compounds from aqueous matrices. The method is based on the same principles as solid phase microextraction (SPME). However, a relatively large amount of extracting phase is coated on a stir bar as compared with SPME. Solutes are extracted into the coating, based upon their octanol–water partition coefficient and upon the sample–sorbent medium phase ratio. The technique has been applied successfully to trace analysis in environmental, biomedical and food analyses [163-165].

Stir bars are coated with a layer of polymer coating, usually polydimethyl siloxane (PDMS), and then used to stir aqueous samples, thereby extracting and concentrating analytes into the PDMS. The coating material used in SBSE is the same as that of SPME, but the amount used is 50 to 250 times greater then SPME. SBSE is by nature an equilibrium extraction technique where the amount of analyte extracted from the aqueous phase is controlled by the partition coefficient of solutes between the sample matrix and coating material.

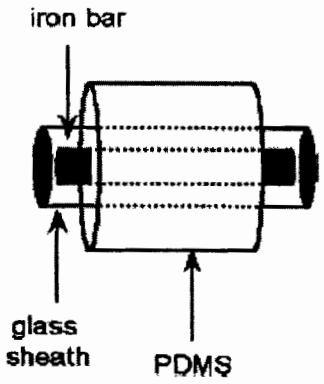
The stir bars are made up of three essential components. The first and innermost is a magnetic stirring rod, which is necessary for rotation above a stirring plate. The second component is a thin layer of glass jacket that covers the magnetic core. This layer is acts as a barrier between the PDMS and the metal to prevent decomposition of the coating. The third and outermost component is the PDMS coating where analytes are extracted. Figure 2.1 shows a Gerstel Twister ™ (Baltimore, MD) and a schematic of a stir bar sorptive extraction device.

PDMS stir bars (Twister™) are commercially available from Gerstel in two configurations, both coated a with 1 mm layer of PDMS: 10 mm L x 3.2 mm o.d. and 40 mm L x 3.2 mm o.d. Typically the 10mm stir bars are used for 1-50 mL sample volumes and the 40 mm stir bars are used for 100-250 mL sample volumes. Because 50 to 300 μL PDMS coatings are used, the sensitivity of this technique is increased by a factor of 100 to 1000 in comparison to SPME.

Sample extraction is performed by placing the sample in an extraction vial, adding a stir bar and magnetically stirring for a suitable period of time. The extraction time, which must be optimized for a given application, is controlled kinetically and is determined by sample volume, stirring speed, and amount of coating material. As in SPME, optimization is done by plotting the extraction time profile to determine the time point where no additional recovery is obtained with additional extraction time. After the equilibrium time point is reached, the stir bar is removed from the sample, and wiped gently to remove water droplets. Where dirty sample have been extracted, it is sometimes recommended to rinse the stir bar slightly with distilled water to remove undesired



(A)



(B)

Figure 2.1 A Gerstel Twister™ stir bar (A), and a schematic of a stir bar showing the three essential components (B).

sample components. Negligible analyte loss occurs during gentle rinsing because the sorbed analytes of interest are present in the polydimethysiloxane phase [166].

In gas chromatography (GC), the analytes are thermally desorbed from the stir bar. Solvent desorption can also be used to remove analytes from the extracting phase thereby enabling the utilization of SBSE with other instrumental techniques such as HPLC.

Quantitation of analytes in SBSE can be accomplished in several ways, primarily governed by sample complexity. Both internal and external standards can be used for clean samples that have minimal matrix interferences. For complex samples such as biological fluids, wastewater and food extracts, internal standards using deuterated analytes and standard additions can be used.

2.2 THERMODYNAMICS

The theory of SBSE is relatively straightforward and similar to that of SPME. The partition coefficient of analytes between the phases is the primary factor that determines the extraction efficiency. Recent studies have correlated the partition coefficient with the octanol-water distribution coefficients ($K_{o/w}$). Although not exactly the same, the octanol-water coefficient gives a good estimation on the ability of the coating to extract an analyte. One of the benefits of using a PDMS phase for sample extraction is that analyte partitioning from water is proportional to the octanol:water partition coefficient. This

allows an estimation of analyte recovery from aqueous solution, and predictions of the approximate detection limits that will be achievable from a given sample size [167].

If the approximation is made that the partition coefficients between PDMS and water ($K_{PDMS/W}$) are proportional to the octanol-water partition coefficients, it can be stated that

$$K_{O/W} \approx K_{PDMS/W} = \frac{C_{SBSE}}{C_W} = \frac{m_{SBSE}}{m_W} X \frac{V_W}{V_{SBSE}} \qquad \text{Equation 2.1}$$

where C_{SBSE} and C_W are the analyte concentration in the SBSE and water phase, respectively, m_{SBSE} and m_W are the mass of analyte in the SBSE and water phase, respectively, and V_{SBSE} and V_W are the volume of the SBSE and water phase, respectively. With the phase ratio defined as β equals V_W/V_{SBSE}, Equation 2.1 can be rewritten as

$$\frac{K_{O/W}}{\beta} = \frac{m_{SBSE}}{m_W} = \frac{m_{SBSE}}{m_0 - m_{SBSE}} \qquad \text{Equation 2.2}$$

where m_0 is the total amount of analyte originally present in the water sample. Equation 2.2 can be rewritten to reflect the extraction efficiency or recovery from the water sample giving

$$\frac{m_{SBSE}}{m_0} = \frac{\left(\frac{k_{o/w}}{\beta}\right)}{1+\left(\frac{k_{o/w}}{\beta}\right)}$$

Equation 2.3

The only parameter controlling the recovery of an analyte from the sample is the ratio of the partition coefficient and the phase ratio between the PDMS on the stir bar and the water sample. Figure 2.2 illustrates the recovery of extraction as a function of the ratio of octanol-water partition coefficient and phase ratio ($K_{O/W}/\beta$). At $K_{O/W}/\beta = 1$ the recovery is 50%. At low $K_{O/W}/\beta$ the values of recovery is approximately equal to the $K_{O/W}/\beta$. At high $K_{O/W}/\beta$ the extraction is essentially quantitative.

In a 100 μm PDMS SPME extraction where the coating volume is 0.5 μL, and a sample volume of 10 mL is used, the phase ratio is 2×10^4. In this scenario quantitative extraction is only achievable for compounds with a $K_{O/W}$ in excess of 10^5. Since only a few compounds have such high $K_{O/W}$ values, it is unlikely that quantitative extraction will occur in SPME. In SBSE on the other hand, the situation is much more favorable to achieve quantitative extraction. A stir bar coated with 100 μL of PDMS, used to extract 10 mL of water exhibits a β of 100, which implies that analytes with a $K_{O/W}$ in excess of 500 are extracted quantitatively by SBSE. This ensures a significantly increased sensitivity for those analytes with a $K_{O/W}$ below 10^5.

Figure 2.3 shows the theoretical recovery of analytes in SBSE and SPME from a 10 mL water sample as a function of their octanol-water partition coefficient. It is obvious that quantitative extraction is obtained at a much lower $K_{O/W}$ in SBSE compared with SPME which is a direct result of the much lower phase ratio.

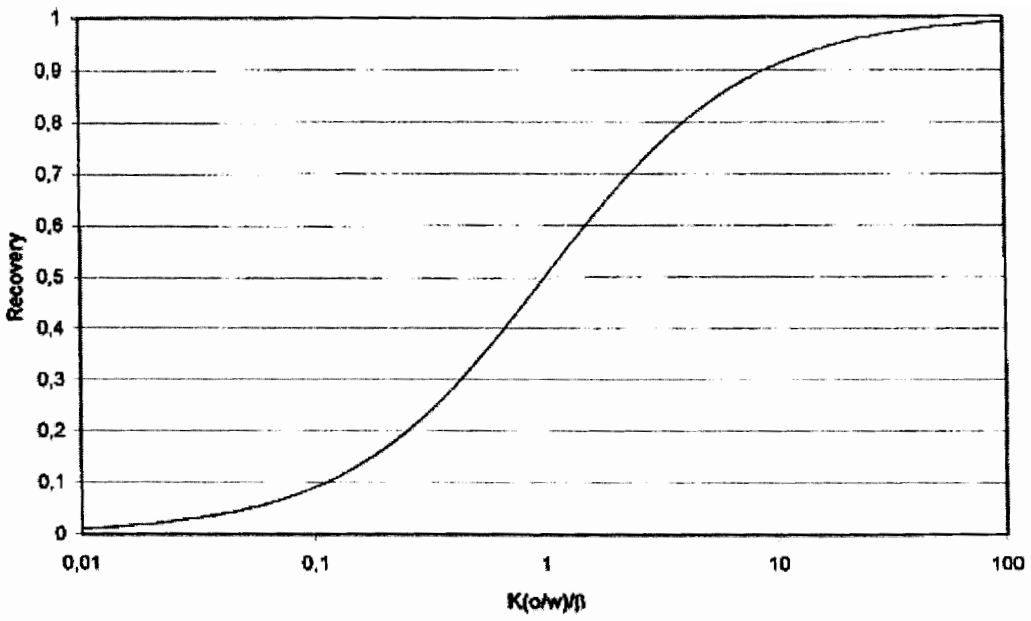


Figure 2.2 Recovery as a function of the ratio of octanol-water partition coefficient and phase ratio ($K_{O/W}/\beta$) for sorptive extraction (adapted from Reference 59).

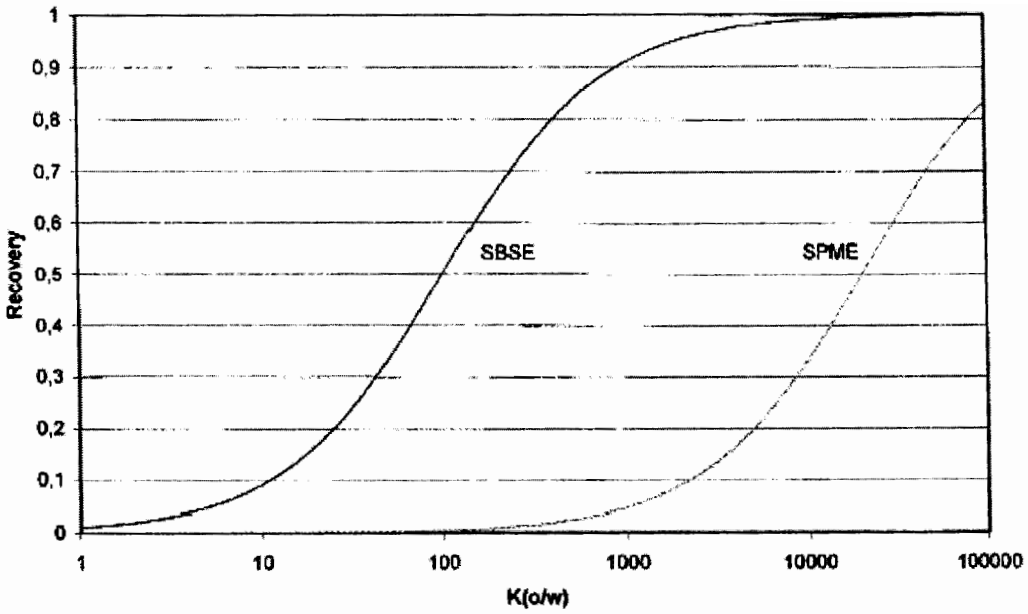


Figure 2.3 Theoretical recovery of analytes in SBSE and SPME from a 10 mL water sample as a function of their octanol-water partition coefficient (0.5 μL PDMS for SPME and 100 μL PDMS for SBSE; adapted from Reference 59).

2.3 KINETICS

The kinetics of extraction in stir bar sorptive extraction are controlled as in SPME. The stirring speed, sample volume, stir bar dimensions and partition coefficient will determine the optimum extraction time. In any method development using SBSE, the extraction time profile should be investigated by extracting a sample under a given set of conditions for progressively longer periods of time and determine the response for each time point. Optimum conditions are achieved when no additional recovery is obtained when the extraction time is increased further.

Since stir bars have much a much larger volume of PDMS than SPME fibers, more materials need to be transferred to the coating phase, and thus more time is required to reach equilibrium. Increasing the stirring speed of the stir bar increases the rate of extraction because more analyte molecules will make contact with the PDMS phase in a given period of time, facilitating absorption.

Although the temperature effects observed in SBSE are not as significant as in SPME, increasing the temperature of the sample during SBSE could potentially reduce the equilibration time by increasing the diffusion coefficient of analytes, However, increasing the temperature will lower the analyte partition coefficient thereby reducing the amount of analyte transferred from the sample to the stir bar coating. At higher temperatures the sensitivity of the SBSE method will be compromised. An elevated sample temperature can be used for extraction if a shorter extraction time is more important than achieving high sensitivity. As in SPME the method should be optimized to achieve the desired sensitivity in the shortest possible time.

A non-equilibrium extraction method can also be used in SBSE since full equilibrium is not necessary for accurate quantitation. However, it is desirable to approach the equilibrium extractable amount to maximize sensitivity and remove the burden of timing the stirring period. Compromised reproducibility in recoveries obtained is also more likely in a non-equilibrium extraction method.

2.4 EXTRACTION PARAMETERS

The various parameters that affect SPME also need to be optimized for stir bar sorptive extraction. The efficiency of extraction in terms of amount extracted and the equilibrium is affected by extraction time, stirring speed, temperature of the sample, pH effects, salting effects and sample volume.

Extraction time. The extraction time necessary to reach equilibration in SBSE can be significantly larger than SPME due to the large amount of coating material on the stir bar. The equilibration time is dependent on the mass transport conditions, nature of the analyte and several other extraction parameters such as stirring rate, temperature, pH etc. In most SBSE optimization scheme, the analyte is allowed to reach equilibrium between the sample and the coating. The extraction time profile is obtained by the same procedure described for SPME. Non-equilibrium conditions can also be used in SBSE when the equilibration time is excessively long and sensitivity of the technique is not of greatest concern.

Stirring rate. Digitally controlled stirrers are ideal for SBSE since it is important that all extractions are performed at the same setting. Extraction is efficient when fast rotational speeds are applied with the equilibration time increasingly decrease as the rpm of the stir bar increases. During stirring, the stir bar should not be moving erratically, but in a stable circular motion to achieve efficient contact and allow rapid mass transfer of analytes into the PDMS. During method development rotation speed studies can be done by carrying out the extraction at various stirring speeds to determine the optimum extraction rate.

Temperature. The enthalpy change and the exothermic effects of SPME described in Section 1.2 also apply for stir bars since the principles of absorption in both techniques are similar. Increasing the temperature of the sample in SBSE increases the extraction rate, but at the same time decrease the distribution constant. If the desired sensitivity is achieved at elevated temperatures, then this temperature should be used in the method to facilitate faster extraction time.

pH. The pH of the sample can be an important factor in SBSE when the analytes of interests are polar compounds, acids or bases. Unlike SPME, in SBSE there is only the nonpolar PDMS coating commercially available and therefore it may be even more important to reduce the polarity of analytes by making pH adjustments. For these analytes changing the pH of the sample matrix determines whether the species exist as an associated or dissociated moiety and thereby offering method selectivity in terms of whether or not the analyte gets extracted. For example, in the PDMS coating, as the pH is lowered, more acidic analyte is present in neutral forms making it more likely to partition

into the coating, resulting in higher sensitivity. The same pH and pK relationships described for SPME also applies for SBSE.

Salting Effects. Increasing the salt concentration of the sample matrix can increase or decrease the amount of analyte extracted by the stir bar. Because PDMS is nonpolar is generally important to first convert the charged analytes into neutral forms. Since the solubilities of many organic compounds in an aqueous solution decrease in the present of excess salt, a simple way to change the solubility of analytes and hence increase the partition coefficient of analytes between the sample matrix and coating is to add salt to the matrix.

Sample volume. Since SBSE is not usually employed for very small sample volumes as in SPME, sample volume is not a critical parameter in the optimization process. The distribution constant determines minimum sample volume that should be used in an SBSE method. Similar to SPME, minimum sample value can be calculated from the K_{fs} or it can be determined experimentally. Typically 10-50 mL sample volumes are used for the 10 mm stir bars and 100-250 mL sample volumes are used for the 40 mm stir bars.

2.5 DESORPTION

In most stir bar sorptive extraction applications, analytes are thermally desorbed from the coating. The optimum temperature for desorption of analytes from the stir bar is analyte dependent. Depending on the concentration and volatility of the analytes,

desorption can be achieved between 150 °C and 300 °C in 5 to 15 minutes under a 10-50 mL/min gas flow. Alternatively analytes can be removed from the stir bars using solvent desorption.

For GC applications, the desorption of analytes from the stir bar is accomplished via a thermal desorption system mounted on top of the instrument. Because more analyte needs to desorbed from the stir bar, the time required for desorption is much longer than SPME. Therefore the desorption process is combined with a cold trapping and re-concentration step for analytes before injection into the column. Two desorption systems are commercially available from Gerstel. Both systems use a programmed-temperature vaporizing injector operated as a cryotrap for cryogenic refocusing of the thermally desorbed analytes. Liquid nitrogen is used for cooling where temperatures as low as -150 °C are used. The desorption units can be fully automated to control the conditions of desorption, trapping and injection.

During method optimization the desorption conditions that are usually optimized include desorption temperature, desorption time and flow rate of the carrier gas. To ensure that complete desorption is occurring, carryover studies can be performed by re-desorbing the stir bar after the initial run. Higher flow rates are more desirable for effective desorption, especially if the analytes or interest are less volatile.

Solvent desorption techniques depend on the type of solvent used, the desorption time, stirring speed and also temperature. Solubility factors are important in solvent selection because the analyte should preferentially partition from the stir bar coating into the solvent. Increasing the temperature and stirring rate can significantly improve the desorption efficiency.

2.6 APPLICATIONS

Stir bar sorptive extraction has been successfully applied to environmental, food, and biological analysis. The many advantages of the technique such as simplicity, solvent-less extraction and excellent sensitivity have made SBSE suitable for field analysis, sample screening and multi-compound analysis. Because of the high inherent enrichment factor, the sensitivity of stir bars in combination with chromatographic techniques such as GC and HPLC is very high.

SBSE is well suited for environmental analysis because it can be applied to volatile organic compounds (VOC) and semivolatile compounds. SBSE has been successfully applied for volatile aromatic, halogenated solvent, polyaromatic hydrocarbon, polychlorinated biphenyl, pesticide, odor compound, organotin compound and phenol analyses [168-174]. Although the majority of these analyses were done with aqueous samples, especially drinking water, SBSE can also be applied for other environmental matrices with appropriate modifications.

It has also been applied to the detection and quantitation of analytes in biological fluids such as urine, serum and plasma. Analytes that have been successfully extracted from these matrices include phenols, steroids, fatty acids, drugs of abuse, barbiturates, and benzodiazepines, phthalates and metabolites [175-177]. Urine samples have been extracted directly or after enzymatic hydrolysis. *In situ* derivatization can also be used for these analyses. More complex samples such as blood, and sperm are usually diluted or pretreated before extraction.

Food analysis is another area where stir bar sorptive extraction has been extensively applied. The technique has been used to extract analytes from alcoholic beverages, dairy, fruits and vegetables. The performance of stir bar extraction for the enrichment of pesticides from fruits, vegetables and baby food was investigated in combination with GC/MS [178]. The multi-residue method developed provided detectabilities from the ppm to the ppb levels. Other applications include preservatives, flavors, contamination and other contents in food products [179,180]. In samples that contain high levels of alcohols or fat content, pre-treatment steps and/or dilutions may be necessary before stir bar sorptive extraction can be performed effectively.

CHAPTER 3

ION MOBILITY SPECTROMETRY

3.1 INTRODUCTION

Ion mobility spectrometry (IMS) is used to characterize chemical substances based on the mobilities of ions in the gas phase. The technique was introduced as an analytical instrument in the 1960's and was initially called plasma chromatography. The development of mobility theory and the emerging analytical applications for IMS were later discussed by Karasek [181]. In the 1970's the number of publications on IMS decreased due to limitations of the technique. However, military establishments in the United States and the United Kingdom continued to develop a base of experience and research with the technique.

During the 1990's, dramatic changes occurred in both the practical aspects of IMS and the understanding of underlying principles of response. IMS instruments designed for on-site monitoring of chemical weapons were successfully distributed in the tens of thousands during the late 1980's and used in battlefield environments in 1992-93. The scale of this application was unprecedented in the history of sophisticated analytical instrumentation. Adaptations of new analyzers and new designs have also occurred for use in industrial and environmental venues, and such developments are particularly encouraging for those interested in comparatively inexpensive yet advanced analyzers.

These changes have not been thoroughly described or explored in recent years and existing reviews are outdated and often incomplete.

Over the past few years, IMS has received a fresh examination, where only a decade ago the technique was regarded largely as a curiosity or an outdated technology within the ion-molecule chemistry and vapor sensing community. The resurgence of IMS instrumentation is due to improved instrument design and also to its intrinsic features of response (excellent detection limits) and to practical considerations (solvent-less nature, size, weight, and power advantages) when compared with well-established technologies such as mass spectrometry or gas chromatography/mass spectrometry.

The term ion mobility spectrometry refers to the method of characterizing chemical substances using gas-phase mobilities of ions in weak electric fields. Normally, mobilities are obtained from the time of drift for ions across a fixed length and electric field where time is referenced to the initial injection of ions from the source region to the drift region. Ion mobilities are characteristic of substances and can provide a rapid means for detecting and identifying vapors [182].

IMS is similar to time-of-flight mass spectrometry. Solid or liquid samples are directly introduced to the analyzer by thermal desorption and the resulting vapors are selectively ionized in a controlled chemical ionization environment to produce molecular ions. The ions in question are generated by atmospheric pressure chemical ionization. Sample material is heated to yield a vapor that is swept into a small drift chamber where a beta radiation source ionizes the molecules. The resulting ions are separated according to size and charge as they accelerate toward the detector. At the detector, each ion generates a specific signal, which is a function of ion mobility.

Ion mobility separates compounds on the basis of their different gas-phase ion mobilities, giving a mobility constant (K), defined in Equation 3.1, where v_d is the drift velocity of the ion and E is the electric field in the spectrometer.

$$K = v_d/E \qquad \text{Eq. 3.1}$$

Mobility constants are usually calculated by measuring the time an ion travels down the drift tube using Equation 3.2, where L is the distance the ions drift, V is the total voltage drop the ion experiences, and t_d is the time it take the ion to travel down the drift tube.

$$K = L^2/Vt_d \qquad \text{Eq. 3.2}$$

It is impossible to separate two compounds with identical K values regardless of the resolving power of an IMS. The ratio of two K values is used to calculate the separation factor (α), defined in Equation 3.3, where K_1 is the mobility constant of the faster drifting ion and K_2 is the mobility of the slower drifting ion.

$$\alpha = K_1/ K_2 \qquad \text{Eq. 3.3}$$

Larger ions have longer drift times than smaller ions as a result of their larger cross sectional areas. The distribution of signals generates an ion spectrum, with an ion mobility band corresponding to each unique ionic species. The spectrum is a fingerprint of the parent compound where molecular characteristics of the sample determine its sensitivity. Analytes may be positively or negatively ionized, alternatively favoring selectivity or sensitivity. The advantages of IMS include low detection limits, simple and reliable instrumentation, and relatively low cost. Vapors from nearly all chemical classes can be ionized either by proton transfer reactions or by negative ion reactions. The technique works well with molecules up to approximately 800 amu, and detection is usually in the high picogram to low nanogram levels. A schematic of an IMS is shown in Figure 3.1.

3.2 Ionization Process

After the samples are injected and vaporized in the desorber unit of the IMS, the analytes are swept into the reaction region of instrument where ionization of the molecules occurs. There are several techniques that can be used to ionize neutral analytes in ion mobility spectrometry. Some of the most common ones reported for IMS are ^{63}Ni ionization, corona discharge ionization and photoionization [183]. The main ionization source used to date is ^{63}Ni due to its high stability, noise-free operation, lack of demand for power, mechanical and physical stability. Ionization occurs in the gas phase by proton or electron transfer where a source of dopant reactant ions are added to facilitate the transfer process. Due to the mild energetics of the atmospheric pressure chemical ionization process, little or no fragmentations of ions are observed.

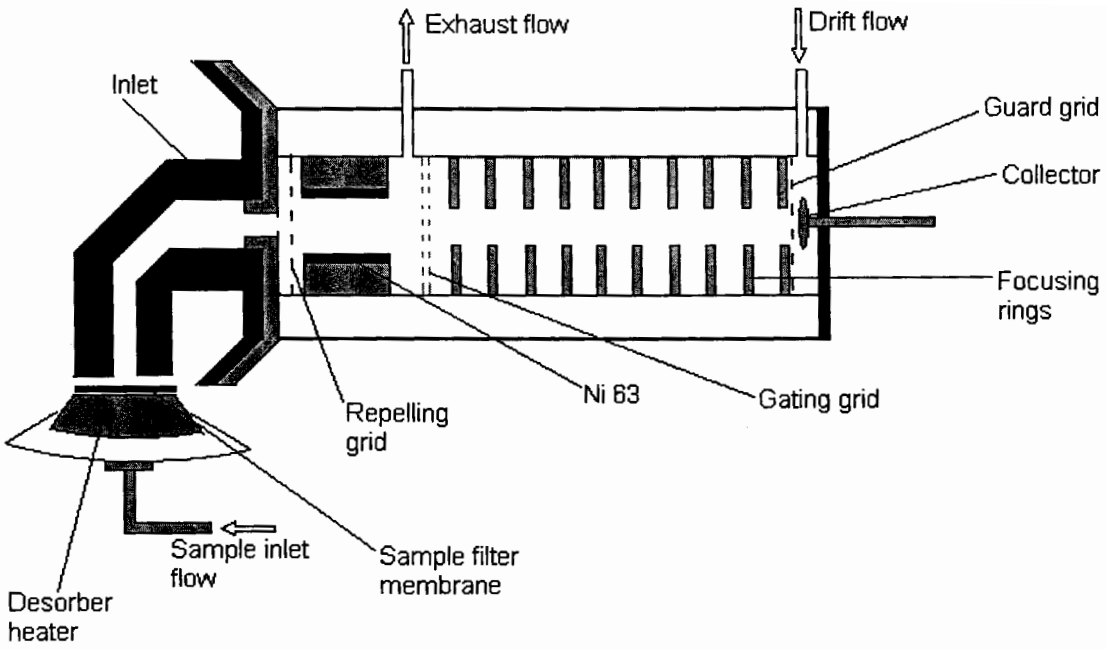


Figure 3.1 Schematic of an ion mobility spectrometer instrument showing the major components of the system

The intensity of the proton or electron transfer reactions depends strongly on the gas phase basicities (electron or proton affinities) of the analytes as well as the type of reactant, instrument temperature and carrier gas used. The ions formed in IMS can be observed isolated or in clusters with neutral molecules such as water, nitrogen or carbon dioxide. The following scheme of equations (Eq. 3.4 – Eq. 3.9) has not been confirmed in air at atmospheric pressure, but it is widely accepted and commonly used to explain the formation of positive ions:

$$^{63}Ni \longrightarrow {}^{63}Cu + {}^{0}_{-1}e \qquad \text{Eq. 3.4}$$

Release of β^- from ^{63}Ni

$$N_2 + {}^{0}_{-1}e \longrightarrow N_2{}^{+} + 2e^{-} \qquad \text{Eq. 3.5}$$

$$N_2^{+} + 2N_2 \rightarrow N_4^{+} + N_2 \qquad \text{Eq. 3.6}$$

$$N_4^{+} + H_2O \rightarrow 2N_2 + H_2O^{+} \qquad \text{Eq. 3.7}$$

$$H_2O^{+} + H_2O \rightarrow H_3O^{+} + OH \qquad \text{Eq. 3.8}$$

$$H_3O^{+} + H_2O + N_2 \rightarrow H^{+}(H_2O)_2 + N_2 \qquad \text{Eq. 3.9}$$

Stable reactant ion

The formation of product ions occurs mainly by collisions between the reactant ions and the sample molecules. Additional product ions may also be produced through association reactions with neutral molecules. Positive product ions are formed primarily through proton transfer reactions as shown in Eq. 3.10. Cluster formation and dimerization reactions are shown in Eq. 3.11 and 3.12, respectively.

$$M + H^+(H_2O) \rightarrow MH^+ + H_2O \quad \text{Eq. 3.10}$$

$$MH^+ + nL \rightarrow MH^+ \cdot L_n \quad \text{Eq. 3.11}$$

$$MH^+ + M \rightarrow M_2H^+ \quad \text{Eq. 3.12}$$

The formation of negative product ions in IMS is similar to the reactions that occur in electron capture detectors [184]. In the negative mode, the electrons produced from Eq 3.4 undergo two main reactions with a proton and analyte:

$$e^- + p^+ \rightarrow \text{neutrals} \quad \text{Eq. 3.13}$$
recombination

$$e^- + M \rightarrow M^- \quad \text{Eq. 3.14}$$
associative electron attachment

If oxygen is present in the reaction region, it could also form clusters with analyte ions resulting in additional peaks present in a plasmagram. Proton abstraction reactions are also alternative routes to negative ion formation. The possible reaction pathways presented above are only a partial depiction of the ionization processes that can occur in IMS. Until further studies are done in this area, the fundamental theory of the ionization reactions at atmospheric pressure in IMS remains vague.

Considering the number of reactions possible and that the charge exchange ionization reactions are competitive in nature, it is not surprising that IMS is not very

effective in characterizing complex mixtures. One tool that is used to achieve better selectivity is the addition of a continuous reservoir of dopant/reactant ion to the reaction region of the IMS drift tube. The reactant ion present with its elevated proton affinity practically eliminates the ionization of substances with proton affinities below that of itself. Therefore complications through interferences are eliminated.

3.3 Ion Separation

Separation of ions in IMS occurs in the drift region of the instrument under the influence of an external electric field. The different forces that act on the ion are: (1) resistance encountered by gas molecules, (2) diffusive forces and (3) the electric field. The mobility (K) of an ion is related to the electric field strength by the ion drift velocity, which is inversely proportional to drift time. Mobility is also related to the collision rate, the temperature, the dimensions of the ion, and the collision integral, where the collision integral is influenced by the size of ions or molecules, structure and their polarizability. Compounds with characteristic mobilities will appear at different drift times, providing a basis for both ion identification and ion separations. The mobility of ions in IMS is given by the Revercomb and Mason equation [185]:

$$K = \left(\frac{3q}{16N}\right)\left(\frac{2\pi}{\mu kT}\right)\left(\frac{1+\alpha}{\Omega_D}\right) \qquad \text{Eq. 3.15}$$

where q is the charge of the ion, N is the density of drift gas molecules, μ is the reduced mass of the ion, k is the Boltzman constant, T is the temperature, α is a correction factor and Ω_D is the average ionic collision cross-section of the ion.

When the instrument operating conditions are held constant, mobility depends only on the charge, reduced mass, and collision cross section. For ions that are much larger than the drift gas molecules, the reduced mass is nearly equal to the drift gas mass, and K varies only with q and Ω. Ion size, shape, and polarizability determine collision cross section.

In addition to the masses of the analytes and their collisional cross section, ion mobilities are considerably influenced by temperature, drift gas and ionic charges. The reduced mobility (K_0) is often used for identification purposes, instead of drift times to correct for instrumental and environmental variations. The reduced mobility is defined by the following equation:

$$K_0 = \left(\frac{d}{txE}\right) * \left(\frac{p}{760}\right) * \left(\frac{273}{T}\right) = (cm^2/Vs) \qquad \text{Eq. 3.16}$$

where d = drift length (cm); t = drift time (sec); E = field strength (V/cm); P = pressure (torr) and T = temperature (K).

Some of the more important parameters that affect the mobility of ions in IMS include the voltage, drift gas and temperature. Increasing the electric field strength will increase the average velocity of an ion. However, increasing the gas density will reduce the velocity since the collisional frequency and kinetic energy loss will increase

proportionally. It has been demonstrated that using different drift gases could modify the separation factor, similar to changing phases in chromatography, such that a particular separation can be optimized by selecting certain drift gases [186]. Temperature has been shown to have both a positive and a negative effect on the velocity of ions in IMS and the overall effect on the reduced mobility ultimately depends on the ion mass [187].

IMS can be used to separate isomers of a compound, because their ions may have different collisional cross sections and therefore exhibit different mobilities. The difference in collisional cross section can arise from differences in geometrical structure and or internal charge distribution. The separation capability of IMS is described using either the *resolving power* or the *peak-to-peak resolution* equations.[188] Both equations are taken from chromatography where resolution, R, is given by Equation 3.17.

$$R = \frac{t_d}{\omega_h} \qquad \text{Equation 3.17}$$

Where t_d is the drift time and ω_h is the temporal full-width-at-half-height (FWHH) for the mobility peak. The peak-to-peak resolution R_{pp} is given by Equation 3.18.

$$R_{pp} = 2\left(\frac{t_{d2} - t_{d1}}{\omega_{b1} + \omega_{b2}}\right) \qquad \text{Equation 3.18}$$

Where t_{d1} and t_{d2} are the drift times for two neighboring ion mobility peaks, and ω_{b1} and ω_{b2} are their temporal full-widths measured at the baseline of the plasmagram.

3.4 Instrument Design

The IMS system can be divided into three main components: (1) the sample introduction component, which is the interface with the ambient atmosphere; (2) the drift tube, where ionization and separation of the ions take place and; (3) the detection component, where data is acquired, analyzed and displayed.

The sample introduction component for a typical IMS instrument shown in Figure 3.1 consists of a desorber unit and an inlet. The heated desorber unit is used to vaporize liquid and solid samples. Analytes present in an organic solvent are injected onto a membrane filter, which is present between the desorber unit and the inlet. Purified dry air is drawn up the desorber unit at flow rates typically between 200 and 500 mL/min to transfer the analyte vapors into the inlet. Both the desorber and the inlet have temperature control capabilities to be able to handle different types of samples.

The drift tube is the most critical component of the IMS, where poor design could lead to improper ionization, poor resolution, and reproducibility. The drift tube is typically divided into the reaction region where ionization takes place and the drift region where separation and detection occurs. At the front end of the drift tube is the ionization region where the common radiation source, ^{63}Ni is found. Typically this source is found as a thin layer of metal strip. The maximum energy of the electrons emitted from the source is 67 keV, with the average being 19 keV. A requirement for a good drift tube design is that all of the ionization reactions should be completed in the reaction region and be stable for traversing the drift tube. Between the reaction region and the drift region is shutter grid, which is used to pulse ions into the drift region.

The drift tube is usually a cylindrical metallic or ceramic tube typically 6 to 8 cm long consisting of focusing rings. An electrical field is applied across the tube with capabilities of switching charges to accommodate either positive or negative ions. The drift tube is also designed to allow gases to flow in the opposite direction that ions move to improve the separation process. At the end of the drift tube is the detector.

The most common detector found in commercially available IMS is the Faraday cup. It is a circular collector plate that is used to measure the ion current in the IMS drift tube. An aperture grid which is placed a few millimeters in front of the detector prevents the buildup of an ion charge on the collector plate, imparts energy to the ions to increase the collection efficiency and also filters out artifacts.

The data output of the IMS consists of a plot of the signal intensity as a function of drift time, known as a plasmagram. Since a single scan may be too noisy for practical use, signal averaging is commonly used. The data from several successive scans are combined to improve signal to noise ratio and to produce a two dimensional plasmagram. A series of segments, each with characteristic ion peak patterns for the sample, can be displayed either as a stacked series of segments in the form of a 3D plasmagram.

3.5 Applications

The attractive features of IMS instruments include portability, simple and rugged design, high sensitivity and short analysis time. These features have led to the use of IMS instruments for a wide range of uses in the military, security applications, environmental

analysis, pharmaceutical analysis and forensic applications. IMS is a rapidly advancing technique with a wide range of applications including the trace detection of narcotics, explosives, toxic industrial chemicals (TICs) and chemical warfare agents. Since 2000, IMS has also seen rapid acceptance in the pharmaceutical manufacturing sector as a means of reducing cleaning verification time relative to other methods-such as high performance liquid chromatography. Most recently, there has been interest in the development of IMS instrumentation and applications for the detection of biological warfare agents and, in the consumer industry, for the detection of specific bacterial pathogens in processed and unprocessed foods [189].

Because IMS is inherently ideal for field analysis, the transfer of established laboratory analytical methods into the field for on site analysis has progressed, especially with the development of smaller and lighter IMS instruments. The integrity of the analytical procedure is also not compromised due to the effective operation of the ion source, drift tube and detector at ambient pressure. Some of these IMS instruments that have been very successful for field analysis include the Chemical Agent Monitor (CAM), Itemiser, IONSCAN, ORION and PD5 [190].

The CAM system manufactured by Smiths Detection is the first example of a large scale deployment of IMS technology into the field. This system is ideal for nerve gas detection in the positive mode without the possibility of producing false positive alarms from interfering compounds present in the atmosphere.

Another area that IMS has made a significant impact is in environmental monitoring where challenges involve getting high quality analytical field data with high sample throughput. IMS is well suited to this application, and can provide high speed,

high quality data while deployed in the field. The flexibility of the IMS inlet can be exploited with a variety of analytical sample types including ambient air samples, soil gas samples, groundwater samples, and organic waste samples. Other fields of applications are semi-conductor industry, monitoring of microbiotics in water, the monitoring of anesthetics in operating rooms and the sensing of petrochemical fuels in soils.

The application of IMS to structural studies of small molecules has also been explored since the discovery of the technique in the 1960s. The coupling of IMS with electrospray (ESI) and MALDI ion sources has opened up exciting possibilities for the study of the conformations and structures of a wide range of biomolecules in the gas-phase, including proteins, peptides and oligonucletoides [191].

CHAPTER 4

SOLID PHASE MICROEXTRACTION COUPLED WITH ION MOBILITY SPECTROMETRY (SPME-IMS) FOR THE ANALYSIS OF EPHEDRINE IN URINE

4.1 ABSTRACT

Solid phase micro-extraction (SPME) coupled with ion mobility spectrometry is demonstrated for the analysis of ephedrine in urine. Since its inception in the 1970's ion mobility spectrometry (IMS) has evolved into a useful technique for laboratories to detect explosives, chemical warfare agents, environment pollutants and increasingly, in detecting drugs of abuse. Ephedrine is extracted directly from urine samples using SPME and the analyte on the fiber is heated by the IMS desorber unit and vaporized into the drift tube. The analytical procedure was optimized for fiber coating selection, extraction temperature, extraction time, sample pH, and analyte desorption temperature. The carryover effects, ion fragmentation characteristics, peak shapes and drift times of ephedrine were also evaluated based on the direct interfacing of SPME to IMS. A limit of detection of 50 ng/mL of ephedrine in urine and a linear range of 3 orders of magnitude were obtained showing that SPME-IMS could potentially be used as an effective method for the rapid screening of drugs in biological samples.

4.2 INTRODUCTION

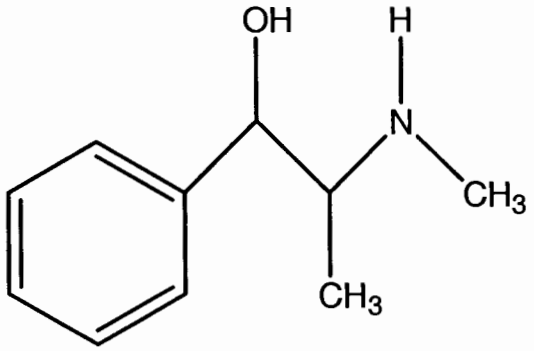
Chinese ephedra (*Ephedra equisetina*), also known as desert herb or Ma Huang, is a plant that grows mainly in Mongolia and the bordering regions of China. Ephedra is a powerful herb that has been used in Chinese medicine for at least 5,000 years. It is the herb from which scientists have extracted ephedrine, one of the most effective treatments known for the symptoms of asthma, allergies, and sinus problems [192]. The active ingredients in ephedra are naturally occurring ephedra alkaloids, the most important one being ephedrine. Because of its stimulating effect on the nervous system, ephedrine can be found in some popular weight loss and energy products. For dieters it suppresses the appetite and stimulates the thyroid gland, which stimulates metabolism.

Ephedra products account for 64 percent of all adverse reactions involving herbs, even though it is found in less than 1 percent of all herbal products sold. The United States Food and Drug Administration (FDA) has reports of over 100 deaths of people who had taken the product. Concerns over the potency of this herb and its isolated alkaloids have prompted increased regulatory scrutiny and industry label warnings [193]. The American Medical Association has also advised people not to use ephedra, which has been banned by the National Football League, the National Collegiate Athletic Association, the International Olympic Committee (IOC) and the World Anti-Doping Agency (WADA), generally establishing a concentration of 10 μg/mL ephedrine in urine, above which is considered a positive doping case [194]. In February 2004 the FDA issued a final ruling, which prohibited the sale of ephedra dietary supplements because they present an unreasonable risk of illness or injury [195].

The popularity and the potentially dangerous consequences of drugs and supplements containing ephedrine has led to a need for their rapid detection and quantitative analysis in biological fluids in field, clinical and forensic laboratory settings. Ephedrine becomes widely distributed by the circulatory system, and is eliminated by first-order kinetics via the urinary system, with analysis of the urine being the primary tool for quantitation of ephedrine in doping cases. These excreted compounds are traditionally analyzed by gas chromatography or high performance liquid chromatography. Both of these methods require time-consuming extensive sample cleanups and long chromatographic run times [196,197]. Derivatization as silyl- or fluoroacetyl derivatives combined with extraction and cleanups may be necessary for separation and quantitation in GC analysis. The problem with gas chromatography-mass spectrometry in the determination of ephedrine is that the reproducibility obtained is not always sufficient, because more than one derivative may be obtained for the same compound [198]. The structure of ephedrine along with the molecular formula and molecular mass is shown in Figure 4.1.

Since its introduction in the 1990's [199] SPME has proliferated with numerous applications where both headspace and direct extraction methods have been thoroughly explored with the primary instrumental techniques being gas and liquid chromatography [207,208]. SPME has previously been used in combination with IMS to detect heroin and cocaine by headspace analysis [209,210]. However, the technique has only been used for semi-quantitative purposes and has not been applied for complex biological matrices.

Ephedrine



Molecular formula: $C_{10}H_{15}NO$

Molecular mass: 165.2

Structural name: (1R, 2S)-2-methylamino-1-phenylpropan-1-ol

Figure 4.1. Structure of ephedrine along with molecular formula, molecular mass and structural name.

Electrospray ionization and matrix-assisted laser desorption/ionization (MALDI) have also been explored as interfacing techniques for SPME with IMS/MS [211,212].

The success of SPME in providing many advantages over conventional analytical methods such as integrating sampling, extraction, and sample concentration into a single step has led us to further explore the technique by coupling it to ion mobility spectrometry to detect and quantify ephedrine in urine. Analysis of ephedrine from urine provides a useful vehicle for exploring the potential for SPME-IMS in the quantitative analysis of drug substances from biological samples.

4.3 EXPERIMENTAL

4.3.1 Chemicals and Materials.

Ephedrine standard and ACS reagent grade acetone were obtained from Sigma (St. Louis, MO). Hydrochloric acid and sodium hydroxide were purchased from JT Baker (Phillipsburg, NJ). Ultra-pure water used in all experiments was obtained from a Milli-Q unit (Millipore, Bedford, MA) installed in the laboratory.

The SPME devices were purchased from Supelco Inc. (Bellefonte, PA) and used with the following extraction fibers: 65 μm polydimethylsiloxane/divinlybenzene (PDMS/DVB), 65 μm Polyacrylate, 7 μm Polydimethylsiloxane (PDMS), 100 μm Polydimethylsiloxane, and 50/30μm Divinylbenzene/Carboxen/PDMS (DVB/CAR/ PDMS). The extraction vials (2 mL) were obtained from VWR (So. Plainfield, NJ). Samples were heated at 80°C using a water bath on a hotplate (Corning 420) during extraction.

4.3.2 IMS PARAMETERS

The ion mobility spectrometer used in this work was the Ionscan LS (Smiths, Warren, NJ) programmed in the positive mode, using nicotinamide as the calibrant/reactant and air as the drift flow gas. The IMS was run with the desorption temperature set at 260°C, the inlet temperature at 260°C, the drift tube temperature at 230°C and the flow rate was set at 300 mL/min. Spectra were collected after a 1 ms delay with a shutter grid width of 0.2 ms. The scan period was set to 30 ms, and desorption time was set at 30 s.

4.3.3 METHODS

A stock solution of ephedrine was prepared at a concentration of 0.2 mg/mL by dissolving 10 mg of ephedrine in 50 mL of purified water. Aliquots of 1 mL urine sample were spiked with various concentrations of ephedrine, and diluted ten-fold with water to reduce matrix interference. The samples were adjusted to pH 10 with 0.5 M sodium hydroxide, using a calibrated pH meter. Aliquots of 2 mL of the spiked urine samples (pH 10) were transferred to vials fitted with PTFE-lined silicone septa. SPME was performed on these solutions using a 50/30 μm Divinylbenzene/Carboxen/PDMS (DVB/CAR/PDMS) fiber, which was conditioned according to the manufacturer's instructions prior to analysis (heated at 270 °C for one hour). The samples were placed in a water bath heated to 80°C by a Corning model 420 hotplate. The SPME fiber was exposed directly into the samples for 30 minutes. The extraction conditions were optimized for coating selection, exposure time, temperature and pH. After sampling, the

fiber was withdrawn into the needle and the SPME device was transferred to the IMS for thermal desorption and final determination. Optimization of each SPME condition was performed using single measurements for each data point.

The extracted analytes were desorbed into the IMS drift tube by depressing the plunger on the SPME holder to expose the fiber. The exposed fiber was placed on the desorption tray in the center of the sampling region, and the tray assembly was slid to the injection port, where the desorber rises, sealing the SPME fiber against the heated IMS inlet. Air was blown through the sampling region at 300 mL/min to transfer the sample into the IMS drift tube for detection.

4.4 RESULTS AND DISCUSSION

Ion mobilities were determined from ion velocities that were measured in the drift tube of the spectrometer. The sample was heated by the desorber unit and vaporized into the instrument inlet. The vaporized sample is conveyed to the reaction region of the spectrometer, by dry purified air, where the reactant ion/calibrant, nicotinamide is added. In the positive ion mode, the gating grid and counter-current drift gas inhibits anions and neutral molecules from entering the drift region of the spectrometer [214].

Ionization selectivity is obtained for compounds whose proton affinities are greater than that of the reactant ion through an equilibrium shift that is determined by the relative proton affinities of the reactant and analyte. In the reaction region, the protonated nicotinamide transfers a proton to the sample molecule according to Equation 4.1. This

reaction only proceeds if the proton affinity of the sample molecule is greater than that of nicotinamide. Most illegal drugs exhibit proton affinities greater than common reactants and are hence readily ionized using this technique.

$$[NTA]H^+ + Analyte \rightarrow NTA + [Analyte]H^+ \qquad \text{Eq. 4.1}$$

For identification purposes, reduced mobilities (K_0) are often used instead of drift time. Reduced mobilities are calculated for positive ion mode using nicotinamide as the calibrant. The reduced mobilities of unknown peaks during analysis can be calculated using Equation 4.2, where K_0 is the reduced mobility in units of $cm^2V^{-1}s^{-1}$ and t is the drift time of the calibrant and the unknown.

$$K_0^{unknown} = \frac{K_0^{calibrant} t^{calibrant}}{t^{unknown}} \qquad \text{Eq. 4.2}$$

Operating at ambient pressures offers many advantages for IMS. These advantages include simple, robust, and miniaturized instrumentation that can be carried out into the field for on site analysis. IMS is also a sensitive technique with real time monitoring capabilities, short analysis times and low detection limits. The consequence of electron transfer is that molecules form ions that maintain much of their original shape and size. Because the energetics of the APCI processes are weak, ions seldom dissociate or fragment in the reaction region of the spectrophotometer.

Different SPME fibers have different selectivities and sensitivities toward the target analyte. Therefore different fibers must first be evaluated and then the optimal one selected for further studies.

Five types of fibers were studied: 65 μm polydimethylsiloxane/divinylbenzene (PDMS/ DVB), 65 μm Polyacrylate, 7 μm Polydimethylsiloxane (PDMS), 100 μm Polydimethylsiloxane, and 50/30 μm Divinylbenzene/Carboxen/PDMS (DVB/CAR/PDMS). The sample concentration of 5 μg/mL and extraction parameters of temperature of 80°C and extraction time of 30 minutes were kept the same for each study conducted on the five fibers. A 2 mL sample volume was also found to be appropriate for the extraction. At volumes greater than 1.5 mL, the amount of analyte extracted by the fiber is independent of the sample volumes. For comparison purposes, the extractions were performed both on urine spiked with ephedrine and also on ephedrine dissolved in water. The recoveries obtained from the urine samples were lower than the aqueous samples due to matrix interference present in the biological media.

Figure 4.2 displays the extraction efficiency of ephedrine in urine and water, by different fiber coatings. This figure shows that the 65 μm PDMS/DVB and 50/30 DVB/CAR/PDMS fibers have comparable responses and exhibit the highest sensitivity to ephedrine, both in water and urine matrix. The difference in extraction performance amongst the fibers reflects variations in the polarity of the individual fibers. The different extraction efficiencies observed are also due the different extraction mechanism of absorption versus adsorption for the liquid PDMS and polyacrylate and the two porous solid fibers.

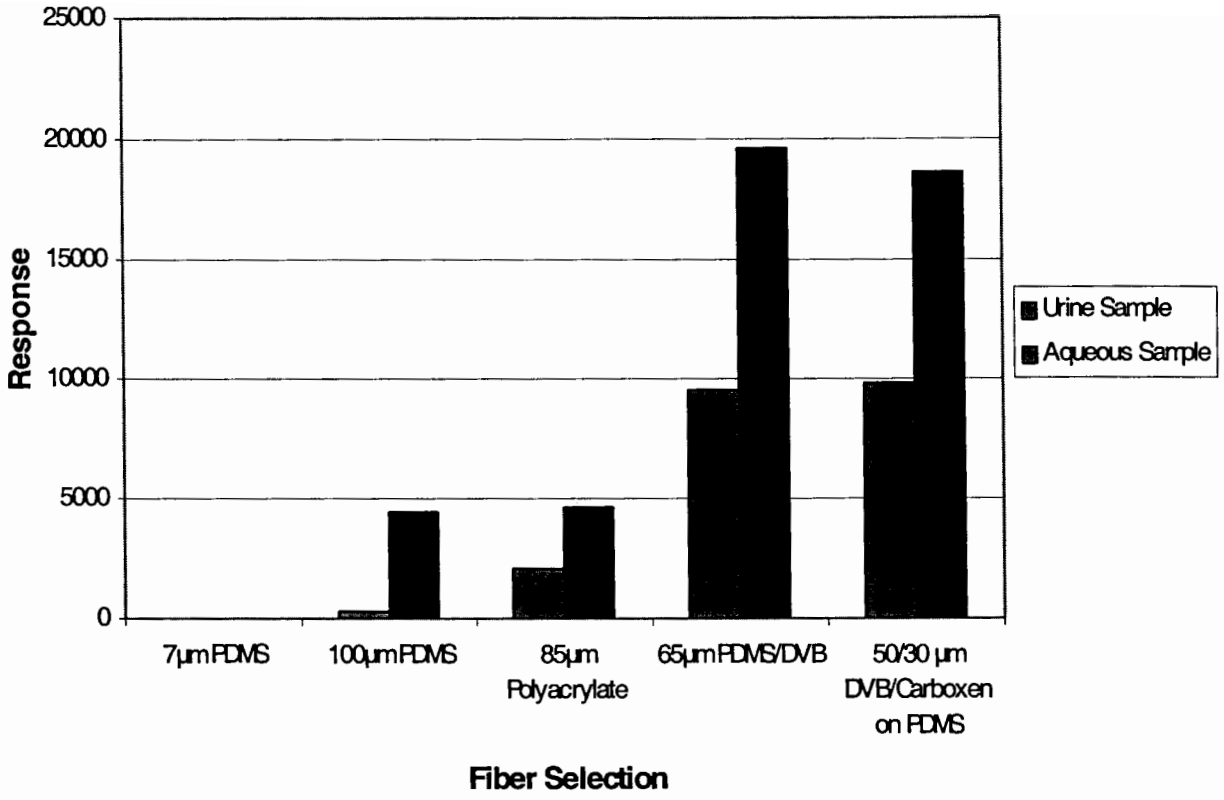


Figure 4.2 Extraction efficiency of ephedrine in water and urine, sampled directly by five SPME fibers

The 7 μm PDMS fiber exhibited poor sensitivity towards ephedrine due to its low polarity and capacity, making it unsuitable for analyzing polar trace level compounds. Compared to the nonpolar 100 μm PDMS and the 65 μm polyacrylate coatings, ephedrine sensitivity could be increased significantly when the PDMS/DVB and DVB/CAR/PDMS fibers are used. The PDMS/DVB and DVB/CAR/PDMS fibers are recommended for polar analytes and hence exhibited higher sensitivity for the highly polar ephedrine compound in comparison with the other fibers. For all further studies, the 50/30 DVB/CAR/PDMS fiber was chosen and the remaining fibers were not investigated further. In this fiber selection study, the extractions were carried out for 30 minutes, and it is possible that equilibrium conditions were not achieved for the PDMS, polyacrylate and PDMS/DVB fibers, since each would have its own equilibration time. The DVB/CAR/PDMS fiber exhibited an equilibrium condition of 30 minutes, as shown in Figure 4.3.

Since SPME is an equilibrium extraction technique, the maximum amount of ephedrine extracted by the fiber under a given set of conditions is determined by the time to reach sorption equilibrium. The efficiency of ephedrine extraction by SPME was investigated using an extraction recovery-time curve for samples. The study was undertaken at 80°C by comparing the response of a known concentration of ephedrine as a function of contact time with the DVB/CAR/PDMS fiber. Six replicate samples were extracted at 5, 10, 15, 30, 45, and 60 minutes. As seen in Figure 4.3, the amount of ephedrine adsorbed to the fiber leveled off after approximately 30 minutes extraction time. Lines drawn across the SPME data points are shown for illustrative purposes. An optimum sample extraction time of 30 minutes was therefore chosen to achieve

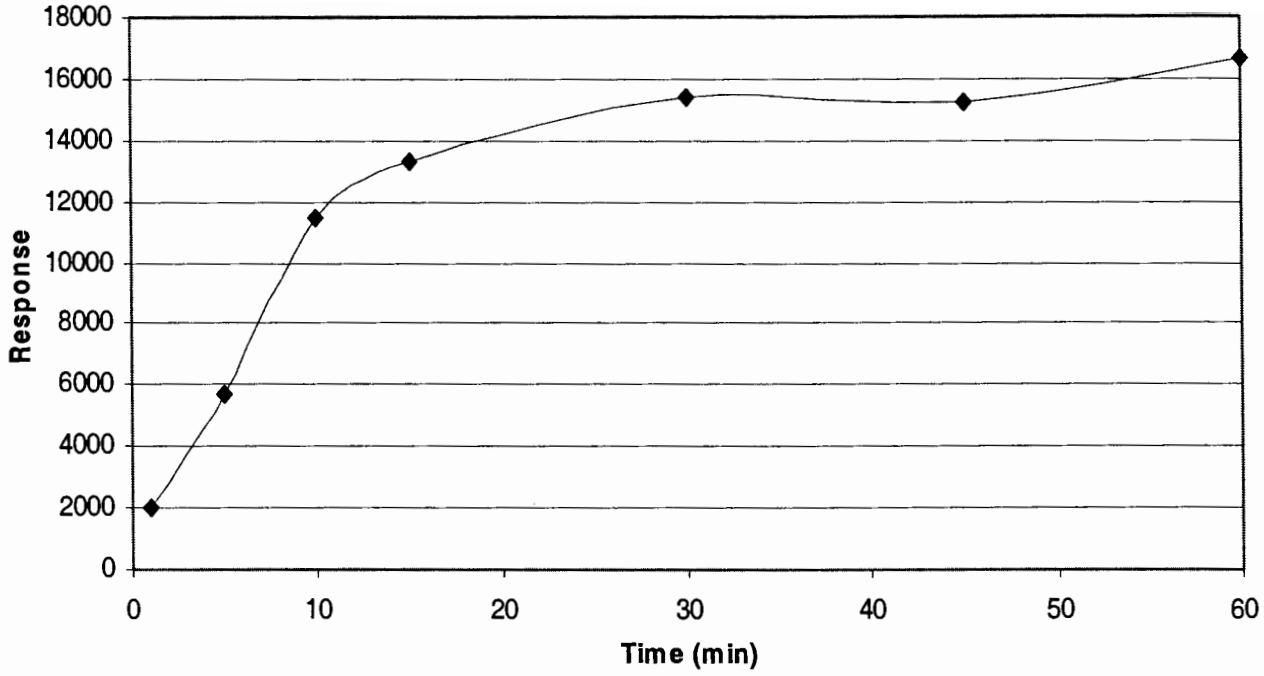


Figure 4.3. Extraction time profile of 5 μg/mL ephedrine solution performed at 80°C with a DVB/CAR/PDMS fiber.

maximum sensitivity without unduly extending the analysis time. Agitating or sonicating the sample during extraction will reduce the extraction time of 30 minutes. However, since this method is proposed primarily for field analysis, it was felt that additional extraction steps would make the method more cumbersome without adding much value, since equilibrium with the DVB/CAR/PDMS fiber was achieved in a reasonable time.

The effect of temperature on the extraction was studied by comparing the response of ephedrine as a function of temperature with the DVB/CAR/PDMS fiber. Four replicate samples were extracted at 25°C, 45°C, 60°C and 80°C. Generally an increase in the temperature of the sample will increase the diffusion coefficient and decrease the distribution constant of analytes, which lead to faster equilibration time and lower extraction recovery.

Figure 4.4 shows that the amount of ephedrine extracted by SPME increases as the temperature is raised and levels off at approximately 80°C. At room temperature the extraction rate is so slow that almost no ephedrine is detected from the fiber after 30 minutes extraction. The extraction rate increases significantly as the temperature is increased to 80°C. At temperatures higher than 80°C, the extraction rate will typically increase as dictated by SPME theory, but the sensitivity of the method will decrease due to a lower distribution constant [215]. A sample extraction temperature of 80°C was therefore chosen for further analysis without compromising the extraction time and sensitivity of the method.

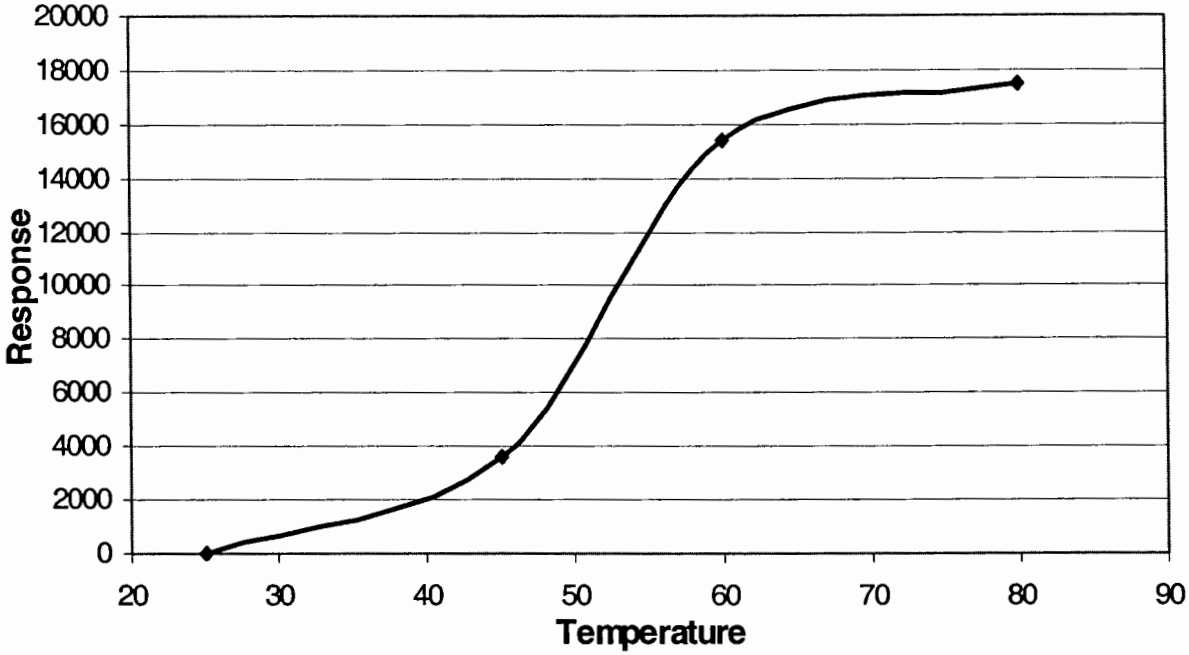


Figure 4.4. Plot of ephedrine response as a function of sample extraction temperature by SPME.

The pH effect on SPME extraction was studied by adjusting the pH of the ephedrine-spiked urine sample with 0.1 M HCl and 0.5 M NaOH. The pH of a urine sample is typically about 6, and the adjustments were made to pH 4, 5, 7, 8, 9 and 10. The study was done by comparing the response of ephedrine as a function of pH with the SPME fiber. Since the pKa of ephedrine is 9.6, the extraction sample should ideally be buffered to pH 10 or 11. Figure 4.5 shows that the amount of ephedrine adsorbed to the fiber increases significantly as the pH is increased from 4 to 10. The manufacturer recommends the maximum pH for immersion of the DVB/CAR/PDMS fiber not exceed pH 11. Therefore, it was decided to continue further studies at a pH of 10 to maximize extraction efficiency while extending the performance of the fiber as long as possible.

According to the manufacturer's instructions, the IMS desorber unit is usually set at 290°C under normal operating conditions where an autosampler is used to make injections. However, since DVB/CAR/PDMS fibers are not stable at such high temperatures, the desorber unit temperature was lowered to preserve the integrity of the fibers. The efficient desorption of ephedrine from the SPME fiber is critical for this technique to achieve good reproducibility, high sensitivity and to prevent excessive carryover between analysis. An SPME-IMS desorption temperature optimization study was conducted to determine the response vs. desorber temperature profile of ephedrine from the DVB/CAR/PDMS fiber. The sample concentration and extraction parameters were kept the same for each of the temperature studies conducted from 200°C to 260°C.

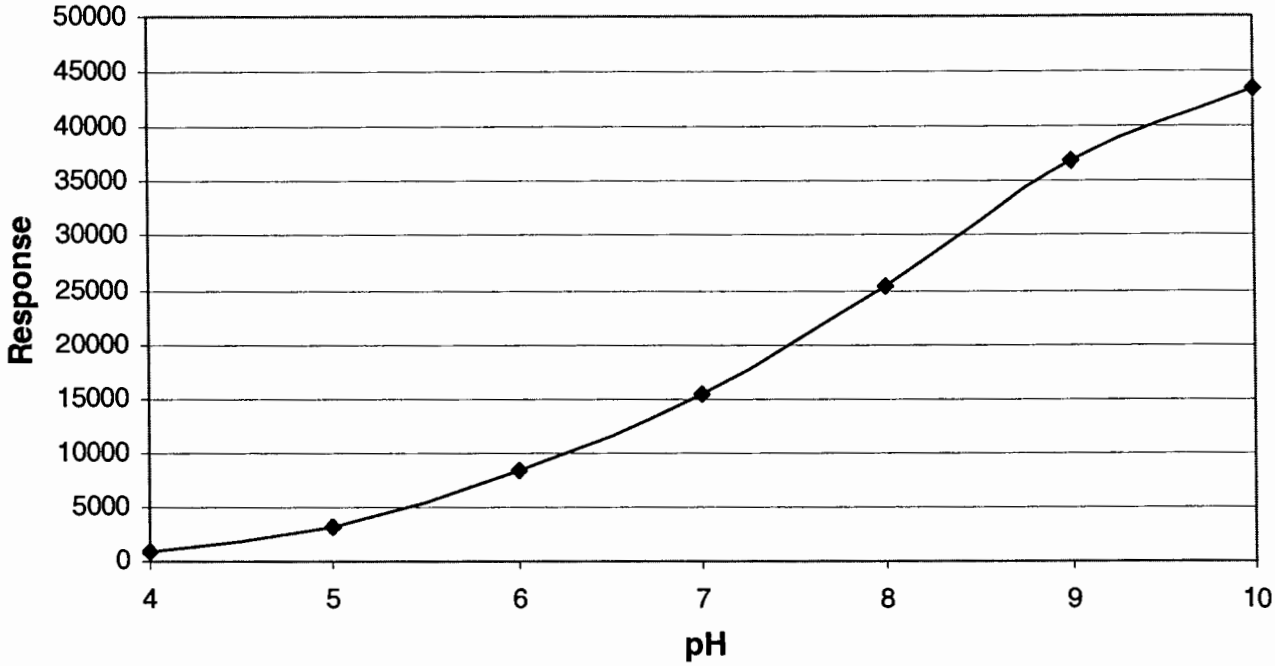


Figure 4.5 Effect of ephedrine response as a function of sample pH by SPME.

The inlet temperature and the drift tube temperature remained constant throughout the experiments at 260°C and 230°C respectively. The SPME carry-over desorption profile was also investigated by making six successive injections from the same extracted fiber to determine the response of ephedrine after each injection. Figure 4.6 shows the results of the desorber temperature profile, and also the carry-over studies conducted on ephedrine in urine samples.

The response of ephedrine increased as the desorber temperature was raised from 200°C to 260°C. As expected, the carryover of analyte also decreased considerably at higher temperatures. At 260°C the carry-over of ephedrine could not be eliminated completely with a single injection and consistently exhibited approximately 3% carryover. The carryover was eliminated completely after 2 subsequent injections. Since each injection require only 30 seconds, it was beneficial to add a cleaning step of two injections to the method after each analysis to ensure no carryover of analytes. Studies were not conducted at temperatures higher than 260°C to preserve the integrity of the DVB/CAR/PDMS coating. The SPME fibers performed well throughout the studies, where over 100 analyses were performed with one fiber before visual degradation of the fiber started to occur. No decomposition products of the fiber were observed in the plasmagrams.

A plasmagram of a blank desorption is shown in Figure 4.7. The only peak present is that of the calibrant nicotinamide at approximately 9.6 ms. the absence of any other peaks indicate that no ionizable substances is desorbing from the SPME fiber.

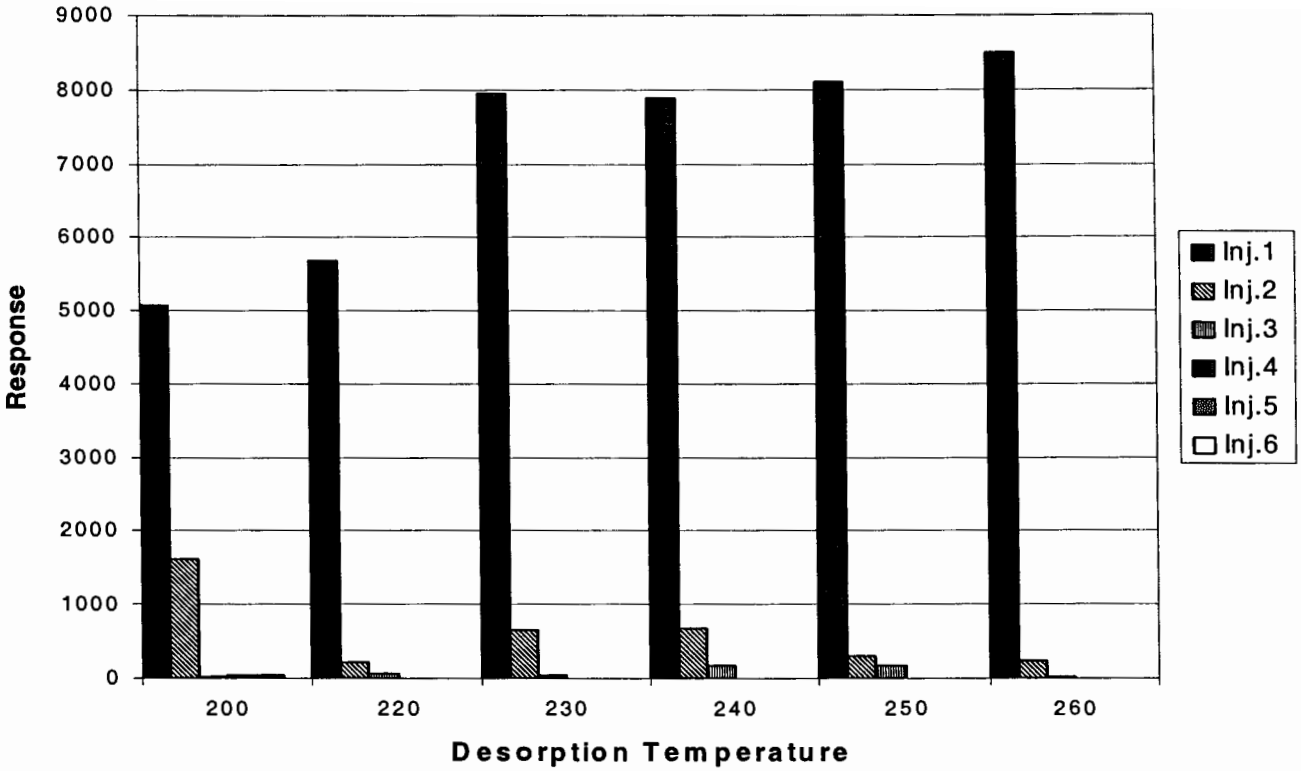


Figure 4.6. SPME-IMS desorber temperature and carry-over profile conducted on ephedrine in urine

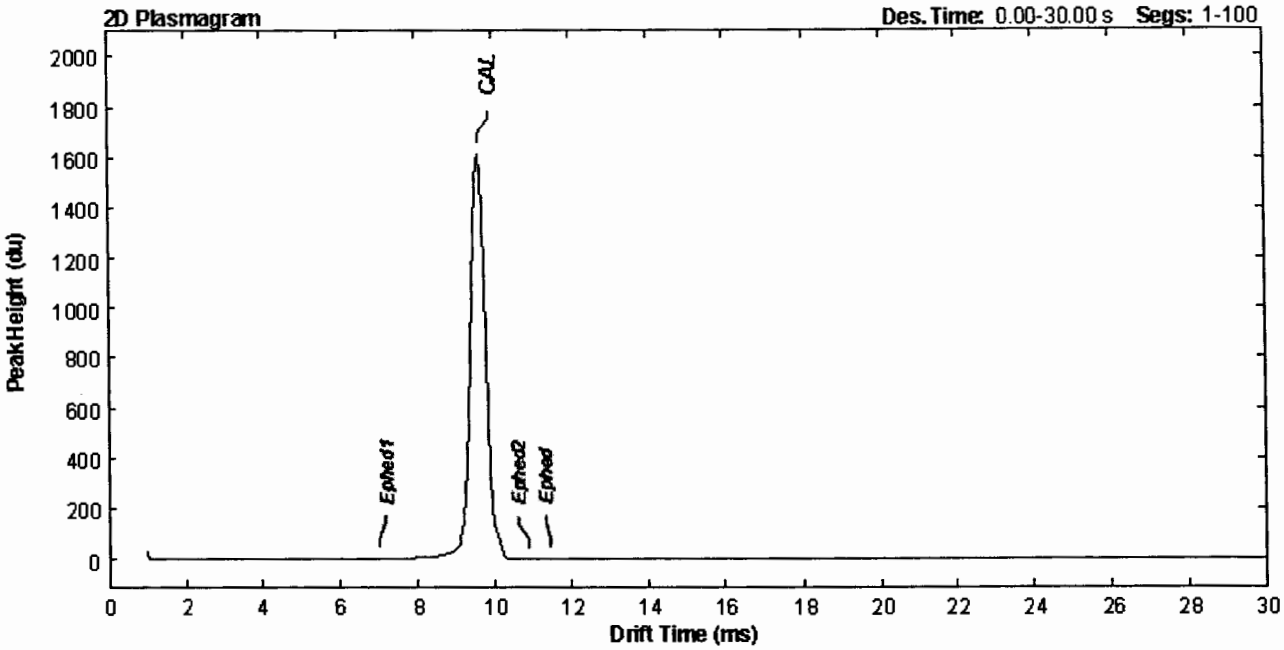


Figure 4.7. A plasmagram of a blank SPME thermal desorption. The only peak present is the calibrant, nicotinamide at 9.6 ms.

A plasmagram of urine sample spiked with 5 μg/mL ephedrine and extracted by the SPME method is shown in Figure 4.8. It was determined from an ephedrine standard prepared in water that the analyte exhibits three characteristic peaks that can be used as a fingerprint tool to identify ephedrine. The primary peak has a drift time of 11.145 ms, and the two secondary peaks labeled as Ephed1 and Ephed2 have drift times of 7.162 ms and 10.620 ms respectively. The peak at 11.145 ms was integrated for all quantitative analysis. The calibrant has a drift time of 9.488 ms. The peak present at 8.755 ms and the smaller ones between 21 and 26 ms are unidentified endogenous substances present in urine.

The fragmentation pattern observed for ephedrine was investigated further by comparing the plasmagrams generated in this study with chemical ionization (CI) mass spectra of ephedrine. While fragmentation is a normal occurrence in electron impact mass spectrometry, the fragmentation of ions in APCI techniques such as chemical ionization mass spectrometry and IMS are considered "soft". Because of this, both IMS and CI mass spectrometry methods exhibit some similar characteristics.

An ephedrine sample, prepared at a concentration of 1 mg/mL in acetone, was injected into a GCQ Chemical Ionization (CI) mass spectrometer (Thermo Finnigan, San Jose, CA) using a high temperature direct injection probe. Methane was used as the calibrant gas and the CI mass spectrum acquired is shown in Figure 4.9 A. As seen in Figure 4.8, ephedrine exhibits three peaks in an IMS plasmagram: a main peaks at 11.124 ms and two secondary peaks at 7.162 ms and 10.620 ms. The chemical ionization mass spectra shown in Figure 4.9 A also exhibits three major peaks at m/z 58, 148 and 166.

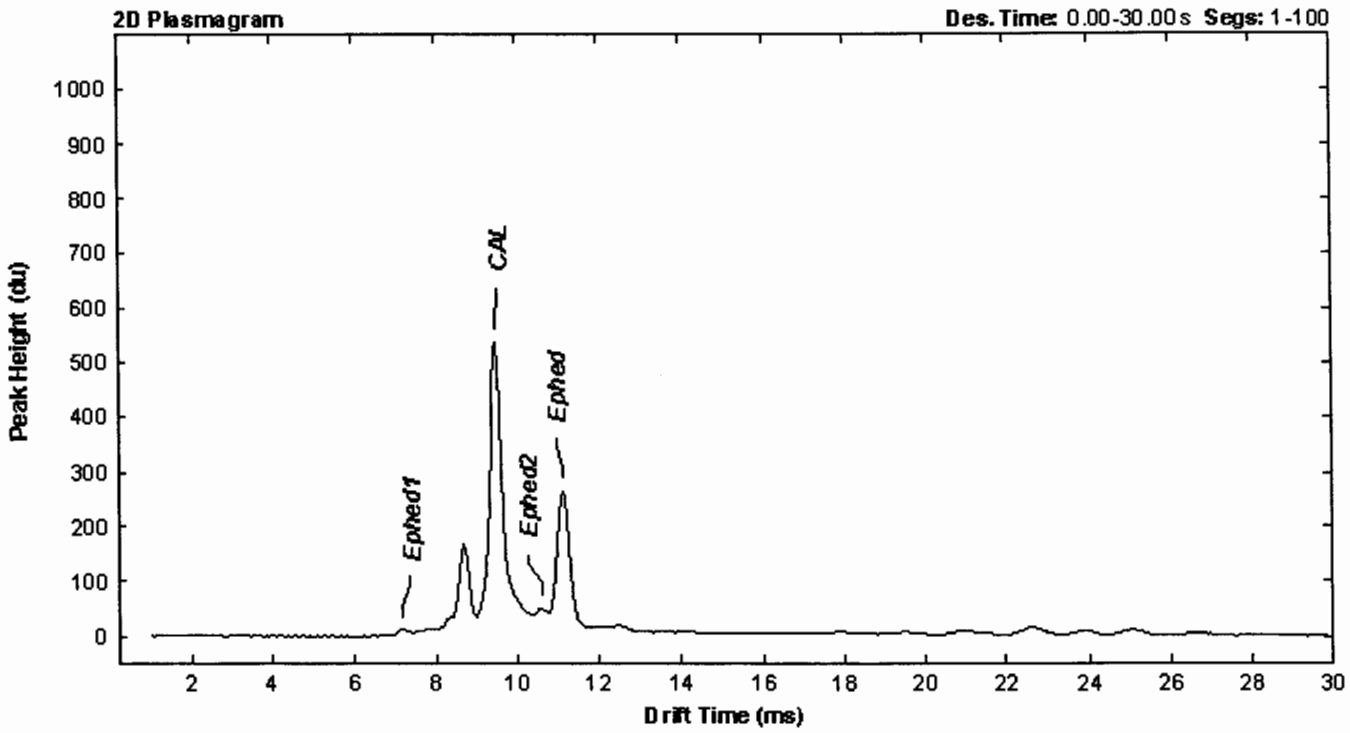
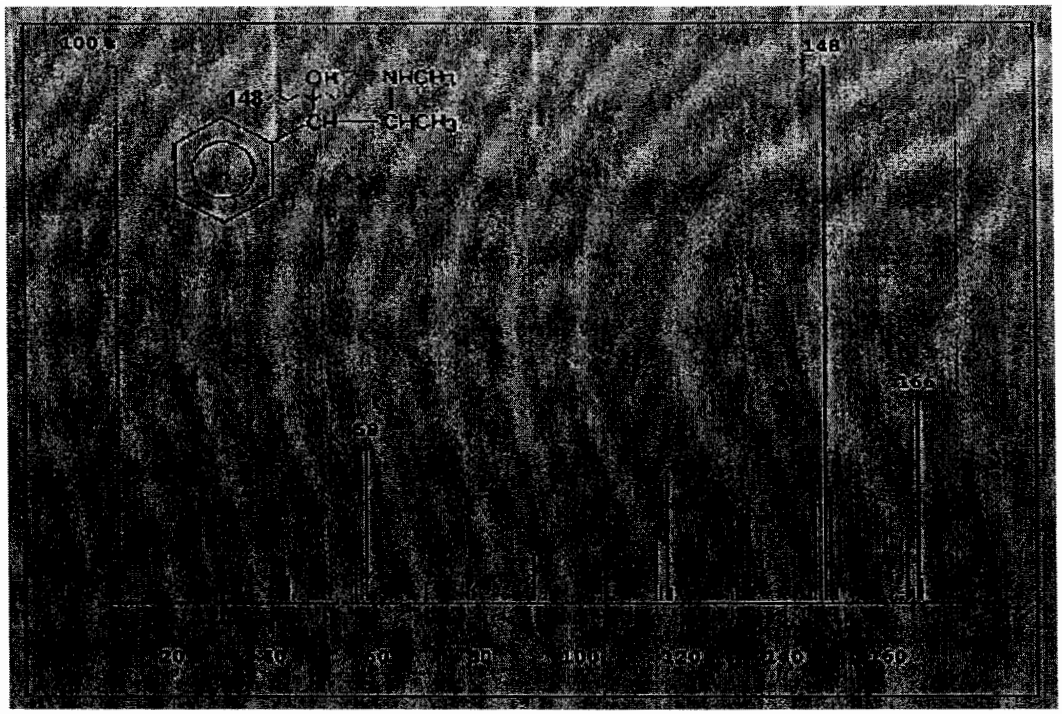


Figure 4.8. A plasmagram of urine sample spiked with 5 μg/mL ephedrine and extracted by SPME

(A)



(B)

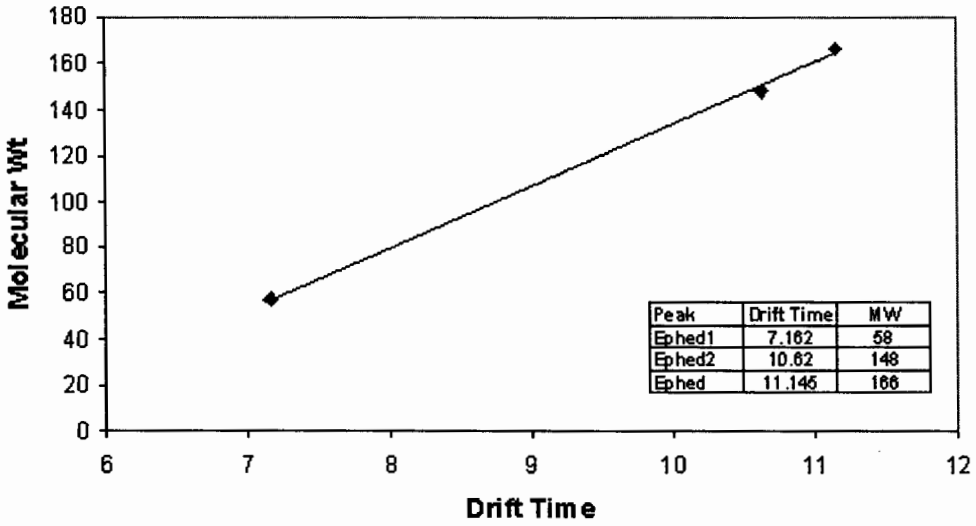


Figure 4.9. (A) Chemical ionization mass spectrum of ephedrine showing the parent protonated ion along with the major fragments at 148 and 58 mz. (B) A plot of drift time v.s molecular weight for the three peaks observed in IMS and CIMS with an r_2 of 0.999.

The peak at 166 is the protonated molecular ion and the remaining two are fragment ions. The molecular structure of ephedrine is also shown in the insert with the carbon-oxygen and the carbon-carbon bonds likely to break and fragment into the associated ions that are observed.

Since IMS is also an APCI technique and it separate ions based on size, it is reasonable to deduce that the peak at 7.162ms is the 58m/z ion, the peak at 10.62 ms is the 148 m/z ion and the primary ephedrine peak at 11.124 ms is the parent ion at 166 m/z. To further substantiate this postulation, a plot of ion molecular weights vs. drift time shown in Figure 4.9 B, yields excellent correlation, with an r^2 of 0.999.

Theses findings support the hypothesis that structural information is encoded on the drift time region of the plasmagrams. This information can be very useful for further studies in the identification of unknown ions produced by an APCI technique by estimating molecular weight of ions base on their drift times in IMS.

A comparison was made of the drift times and peak shapes of ephedrine using direct injection vs. SPME injection was done. A 2 μL sample of ephedrine dissolved in acetone was directly injected into the IMS and compared with the results obtained from an SPME injection. Table 4-1 shows that the SPME injection produces excellent replication of drift times compared with direct injection. The peak-width-at-half-height (FWHM), which is a common measure of peak shape, also shows excellent comparison between direct and SPME injection.

	Direct Injection			SPME Injection		
	Drift Time *(m sec.)*	**K_0** *($cm^2V^{-1}s^{-1}$)*	**FWHM** *(μsec.)*	**Drift Time** *(m sec.)*	**K_0** *($cm^2V^{-1}s^{-1}$)*	**FWHM** *(μsec.)*
Ephedrine	11.145	1.5848	312	11.143	1.5843	308
Ephed1	7.162	2.4660	217	7.161	2.4660	216
Ephed2	10.620	1.6630	244	10.619	1.6630	240

Table 4-1. Comparison of drift time and FWHM between SPME-IMS and direct injection IMS.

Figure 4.10 shows a three dimensional plasmagram of the blank desorption shown in Figure 4.7, with nicotinamide abundant across the 30 second desorption. A three-dimensional (3D) representation of the urine sample spiked with 5 μg/mL ephedrine same IMS plasmagram depicted in Figure 4.11, along with all of the segments of data acquired during the run. When a sample is introduced into the reaction region, charge transfers from the reactant ion to the product ion, which results in a decrease of the reactant ion (nicotinamide) and an increase in the analyte (ephedrine) peaks. As the sample is removed from the reactant region, reactant ions increase and product ions decrease in concentration, resulting in an increase in the reactant ion peak and a decrease in the analyte peak. Figure 4.12 depicts a concentration profile that shows the growth and decay of nicotinamide and ephedrine in relation to each other during the course of the 30 s analysis.

The reproducibility of the method with 1 μg/mL spiked urine sample was evaluated and found to have an RSD (N=5) of approximately 5%. The limit of detection of the method, with the inherent ten fold dilution of urine, was estimated to be 0.05 μg/mL, which is well below the limit of 10 μg/mL set by the WADA and IOC.

Since urine concentration can be highly variable with respect to matrix constituents, the accurate quantitation of ephedrine in urine requires a method of standard addition. In this technique, known quantities of ephedrine are added to the unknown, and the enhanced response allows determination of how much analyte was present in the original urine sample. The method of standard addition mandates a linear response to

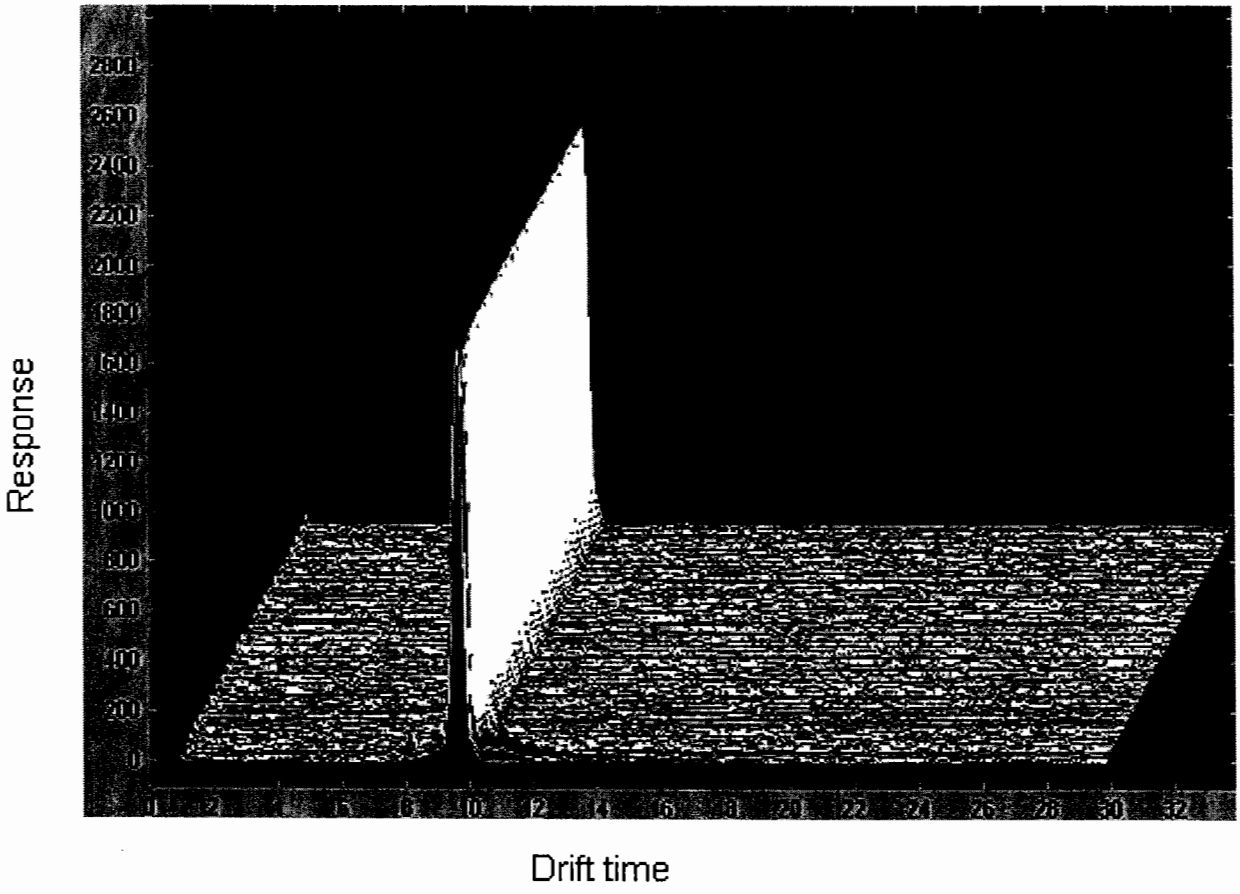


Figure 4-10. A three dimensional plasmagram of a blank SPME desorption.

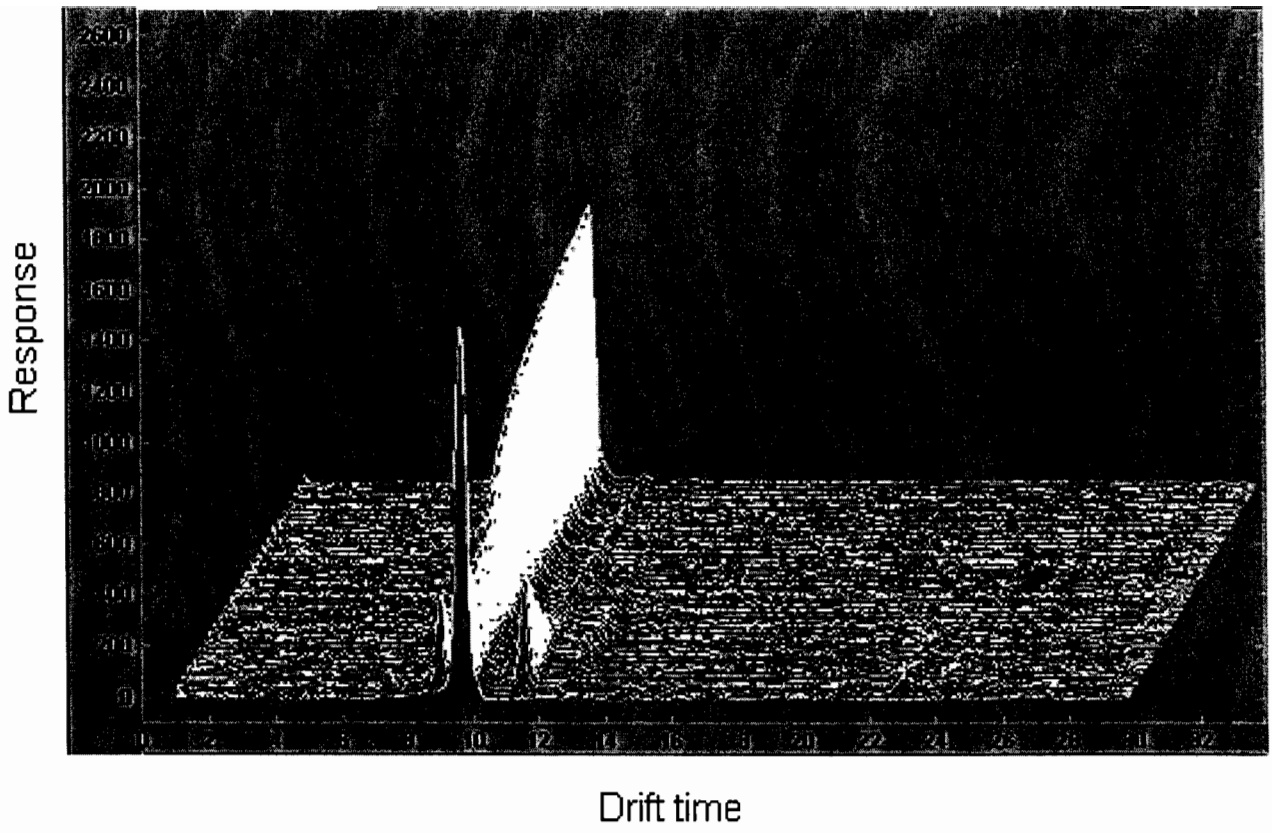


Figure 4.11. A three-dimensional plasmagram showing 5 μg/mL ephedrine spiked in urine

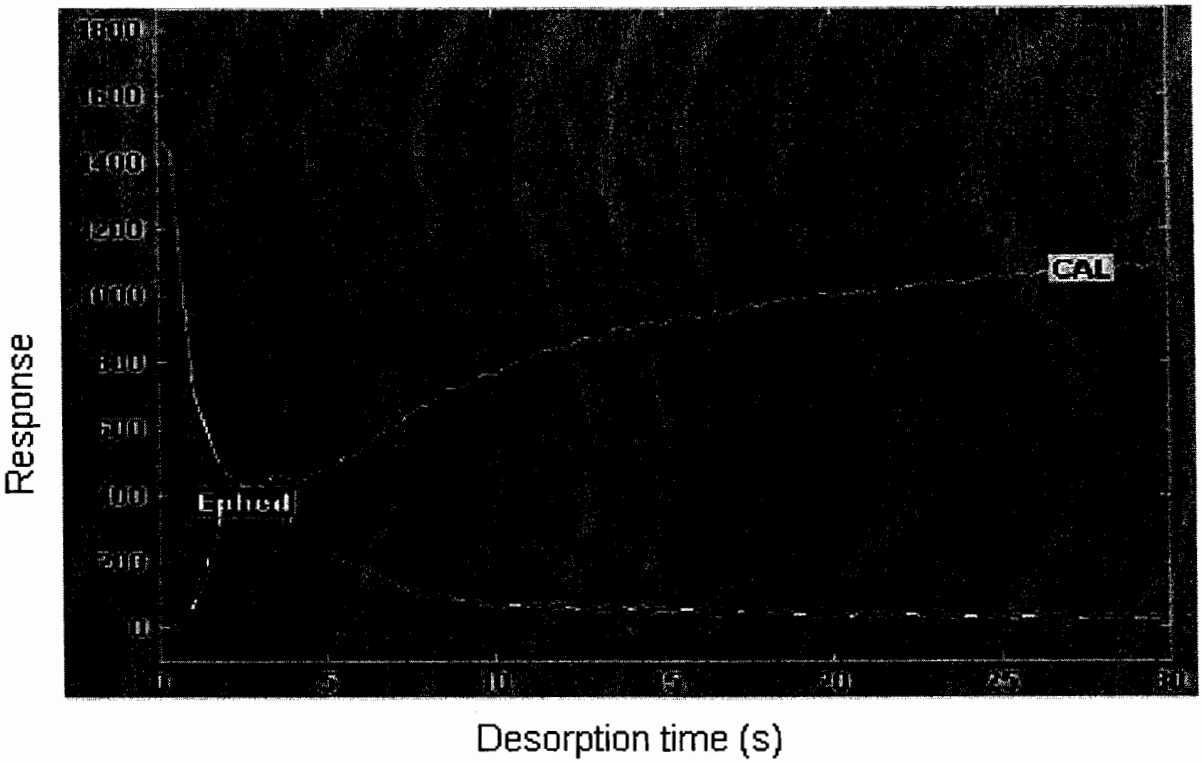


Figure 4.12. A cross sectional view of a plasmagram showing 5 μg/mL ephedrine spiked in urine. The two plots show the desorption profile of the calibrant and ephedrine for 30 seconds

ephedrine, so a calibration curve for SPME-IMS of ephedrine from urine was plotted. The calibration curve, shown in Figure 4.13, was determined in the concentration range of 0.1 μg/mL to 10 μg/mL and found to be linear over the entire range with an r^2 value of 0.990.

A urine sample was spiked with 2 μg/mL ephedrine and analyzed using the method of standard additions. The quantitation of ephedrine in this sample was done by performing a linear regression analysis on the spiked sample with the equation, $y = 3556x + 7542$. The linearity of the calibration curve obtained from this analysis exhibited an r^2 value of 0.992. The recovery obtained on the spiked urine sample was 101%, confirming the applicability of the proposed method for the analysis of ephedrine in urine. This procedure would work well as a screening technique because of its portability, real-time and on-site monitoring capabilities to detect and quantitate ephedrine in urine.

Coupling SPME with IMS produces a system not only with improved sensitivity, but also enhanced selectivity. The additional selectivity provided by SPME, which includes the removal of interferences, is critical for IMS operation in order to circumvent ion competition and suppression, which historically has been the major limitation of IMS instruments to detect and quantitate analytes in complex samples.

The SPME-IMS method developed in this study demonstrates that ephedrine analysis in biological matrices can be done in minutes with minimum sample cleanups and no solvents. The SPME-IMS combination possesses several attractive attributes such as the simplicity of sample preparation, minimized sample handling, and 1-step sample extraction/concentration directly from the biological sample into the IMS.

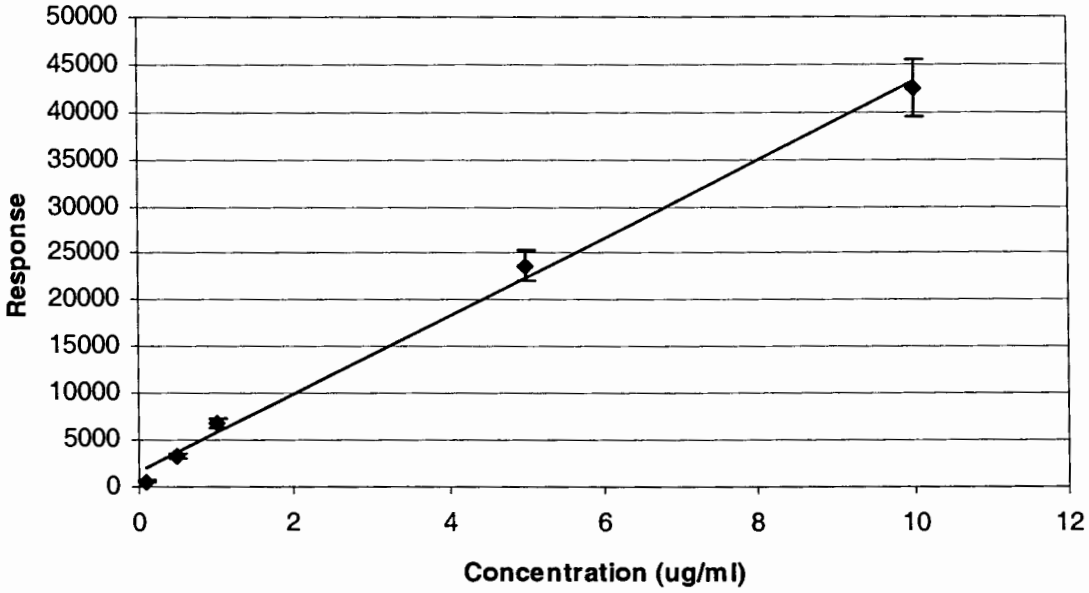


Figure 4.13. Calibration curve for 0.1 μg/mL to 10 μg/mL ephedrine in urine sample extracted by SPME. The equation of the line is $y = 4164x + 1524$ with an r^2 value of 0.990.

4.5 CONCLUSION

SPME-IMS does not require elaborate sample preparation or chromatographic separation and provides a fast, sensitive and reliable method for detecting and quantifying ephedrine in a biological matrix. Although we have not experienced any interference by coexisting substances in quantitative ephedrine detection, confirmation analysis by GC-MS should be performed for IMS-positive samples containing multiple drug substances at high concentrations. The linearity for ephedrine observed over three orders of magnitude, along with a detection limit of 0.05 μg/mL, reproducibility of 5% and good recoveries make the method very satisfactory. The short analysis time, minimization of carry-over, fragmentation characteristics, and field-analysis capabilities also render the method very practical for many applications. However, further evaluation of this technique against well understood and established methods is necessary before SPME-IMS can be solely relied upon for routine measurements.

Method development for SPME-IMS follows similar procedures as for the more common SPME-GC. The SPME-IMS technique could be easily applied to field measurements, since it does not require gases or mobile phase, and the IMS operates at atmospheric pressure. The IMS fragmentation spectra can also be used to give additional evidence for the presence of ephedrine. The limit of detection is well below the cut-off limit specified by the World Anti-Doping Agency, and the linear range, reproducibility, recovery and lack of interference from endogenous compounds make the SPME-IMS method very attractive and promising for routine analysis.

CHAPTER 5

DETECTION AND QUANTITATION OF THE PARABENS IN PHARMACEUTICAL FORMULATIONS BY SOLID PHASE MICRO EXTRACTION/ION MOBILITY SPECTROMETRY

5.1 ABSTRACT

Solid phase micro-extraction (SPME) coupled with ion mobility spectrometry (IMS) is demonstrated for the detection and quantitation of 4-hydroxybenzoate preservatives, methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP) in commercial pharmaceutical products. Since its inception in the 1970's IMS has evolved into a useful technique for laboratories to detect explosives, chemical warfare agents, environment pollutants and increasingly in detecting drugs of abuse. For the first time, an SPME-IMS technique is described for the simultaneous detection, separation and quantitation of multiple analytes in complex matrices. The parabens are extracted from the samples using SPME and the analytes on the fiber are heated by the IMS desorber unit and vaporized into the drift tube. The four preservatives differing only by a methyl group were separated in less than 18 ms. The analytical procedure was optimized for fiber coating selection, extraction time, sample pH, sample volume, ionic strength, and IMS conditions. Separation characteristics such as resolution, theoretical plates, and drift times of the parabens were also evaluated based on the direct interfacing of SPME to IMS. The optimized method was further verified by testing six

over-the-counter topical products containing various combinations of preservatives. Analysis of the samples by SPME-IMS using benzyl paraben as an internal standard yields good comparison to an HPLC method, thereby reinforcing the applicability of this technique as a method for routine analysis. Limits of detection were 10 ng/mL for methyl paraben and ethyl paraben, and 5 ng/mL for propyl paraben and butyl paraben. A linear range of 3 orders of magnitude, and acceptable reproducibility were obtained.

5.2 INTRODUCTION

Parabens are the most commonly used preservatives in topical pharmaceutical preparations. A paraben mix is a mixture of four different paraben esters: methyl-, ethyl-, propyl-, butyl-parahydroxybenzoic acids. They are also used in cosmetics, skin care products, medications, foods, and industrially in oils, fats, shoe polishes, textiles and glues. Two or more paraben esters are often found in one product so it is useful to test sensitivity with the paraben mix, as there is a high incidence of cross-reactions between the esters.

Leave-on products such as facial makeup and skin lotions are of great concern because of the long exposure time and opportunity for migration via the skin into the bloodstream. The recently discovered estrogenic effects of certain synthetic chemicals, including the parabens, and their subsequent effects on the endocrine system of humans and wildlife, is of growing concern, especially in relation to women's risk of breast cancer [216,217]. For many years, parabens were considered among those preservatives with low systemic toxicity, primarily causing allergic reactions. However, as people have

become aware that some synthetic chemicals mimic the female hormone estrogen, the understanding of the toxic effects of both synthetic and natural substances has changed.

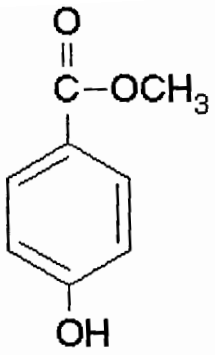
Significant effort has been invested to develop analytical techniques for drugs in clinical, forensic and pharmaceutical applications. These methods often require extraordinary care in sample collection, preparation, and analysis since only small amounts of substances might be available in complex matrices and they are difficult to extract or isolate. Such analyses may be complicated further by chemically interfering components and widely differing sample purities. The demand also exists for rapid, inexpensive, and convenient analytical methods in quality control and quality assurance during the manufacture of drugs. More efficient methods of analysis could expedite the process of performing production line monitoring, spot and final testing of raw materials and finished commercial products.

The popularity of the parabens as preservatives in pharmaceutical formulations has led to a regulatory requirement for their rapid detection and quantitative analysis in development, manufacturing and quality control laboratories. Parabens are traditionally analyzed by gas chromatography (GC) or high performance liquid chromatography (HPLC), with both of these methods requiring time-consuming and extensive extraction, sample cleanups and long run times [218-222]. Derivatization as silyl- or fluoroacetyl derivatives combined with extraction and cleanups may be necessary for separation and quantitation in GC analysis. HPLC techniques, most commonly used for these compounds, require time-consuming extraction steps and long run times involving gradient elution to remove the many nonpolar compounds typically used in topical formulations. There has also been limited use of electrophoretic methods for these assays.

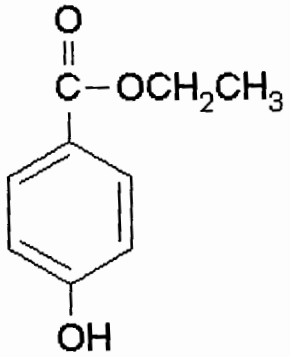
[223-226]. The structures of the parabens used in this study, along with molecular formula and molecular mass information are shown in Figure 5.1.

Since its introduction in the 1990's [227] SPME has proliferated with numerous applications in the forensic, environmental, food and pharmaceutical industry, where both headspace and direct extraction methods have been thoroughly explored with the primary instrumental techniques being gas chromatography and liquid chromatography [228-236]. SPME has previously been used in combination with IMS to detect heroin and cocaine by headspace analysis [237,238]. However, the technique has only been used for semi-quantitative purposes and has not been applied for multi-component analytes in complex samples. Electrospray ionization and matrix-assisted laser desorption/ionization (MALDI) have also been explored as interfacing techniques for SPME with IMS/MS [239,240].

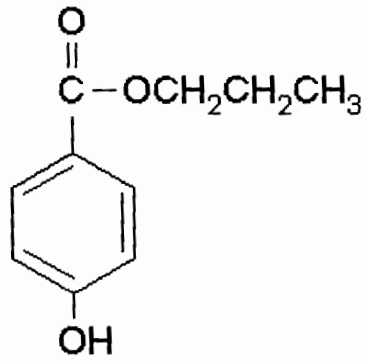
The success of SPME in providing many advantages over conventional analytical methods such as selectivity, and integrating sampling, extraction, and concentration into a single step has led us to further explore the technique by coupling it to IMS to detect and quantify parabens in creams, lotions, solutions and ointments. IMS offers an alternative to the costly and time-consuming traditional chromatographic techniques, and has the advantages of fast, sensitive, real-time and on-site in-process type monitoring capabilities. The SPME-IMS method developed in this study demonstrates that paraben analysis in pharmaceutical products can be done in minutes with minimum sample cleanups and no solvents. The SPME-IMS combination possesses several attractive attributes such as the simplicity of sample preparation, minimized sample handling, and one-step sample extraction/concentration directly from the sample into the IMS.



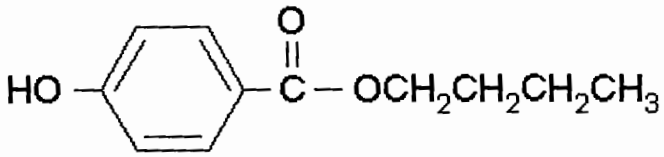
Methylparaben
Structural name: Methyl 4-hydroxybenzoate
Molecular Formula: $C_8H_8O_3$
Molecular Weight: 152.15



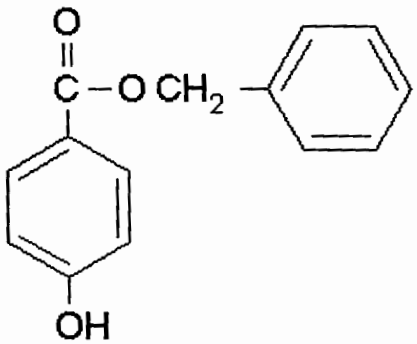
Ethylparaben
Structural name: Ethyl 4-hydroxybenzoate
Molecular Formula: $C_9H_{10}O_3$
Formula Weight: 166.17



Propylparaben
Structural name: Propyl 4-hydroxybenzoate
Molecular formula: $C_{10}H_{12}O_3$
Formula Weight: 180.20



Butylparaben
Structural name: Butyl 4-hydroxybenzoate
Molecular formula: $C_{11}H_{14}O_3$
Formula Weight: 194.23



Benzylparaben
Structural name: Benzyl 4-hydroxybenzoate
Molecular formula: $HOC_6H_4CO_2CH_2C_6H_5$
Formula weight: 228.24

Figure 5:1. Structures of methylparaben, ethylparaben, propylparaben, butylparaben and benzylparaben shown with the structural name, molecular formula and molecular mass.

5.3 EXPERIMENTAL

5.3.1 Chemicals and Materials

Methyl paraben, ethyl paraben, propyl paraben, butyl paraben and benzyl paraben standards were obtained from Sigma (St. Louis, MO). Hydrochloric acid, sodium hydroxide, sodium chloride and ACS reagent grade acetonitrile were purchased from JT Baker (Phillipsburg, NJ). Ultra-pure water used in all experiments was obtained from a Milli-Q unit (Millipore, Bedford, MA) installed in the laboratory.

The SPME devices were purchased from Supelco Inc. (Bellefonte, PA) and used with the following extraction fibers: 65 μm polydimethylsiloxane/divinyl benzene (PDMS/DVB), 65 μm Polyacrylate, 7 μm Polydimethylsiloxane (PDMS), 100 μm Polydimethylsiloxane, and 50/30 μm Divinylbenzene/ Carboxen/PDMS (DVB/CAR/PDMS). The extraction vials (5mL) were obtained from VWR (So. Plainfield, NJ). The over-the-counter topical products, including three creams, a lotion, a solution and an ointment were purchased from neighborhood pharmacies. For the HPLC analysis, a Symmetry ® C18 5μm 3.9 X 250 cm HPLC column was purchased from Waters Corp. (Milford, MA).

5.3.2 IMS Parameters

The ion mobility spectrometer used in this work was the Ionscan LS (Smiths, Warren, NJ) programmed in the negative mode, using hexachloroethane as the

reactant/dopant, 4-nito-benzylnitrile as the calibrant and purified air as the drift flow gas. The IMS was run with the desorption temperature set at 270°C, the inlet temperature at 270°C, the drift tube temperature at 115°C and the flow rate was set at 400cc/min. Spectra were collected after a 1 ms delay with a shutter grid width of 0.2 ms. The scan period was set to 30 ms, and desorption time was set at 30 s.

5.3.3 Methods

Standard stock solutions of the four parabens were prepared at a concentration of 0.1 mg/mL by dissolving 10 mg of MP, EP, PP and BP in 2 mL of methanol and diluting to 100 mL with purified water. The internal standard, benzyl paraben, was prepared similarly at a concentration of 0.1 mg/mL. The working standard solution was prepared by adding 50 µL stock standard and 25 µL of internal standard solution into 10 mL flask and brought up to volume with water, yielding a concentration of 0.5 µg/mL parabens and 0.25 µg/mL of internal standard. Aliquots of 3 mL of the standard solution were transferred to 4 mL vials fitted with PTFE-lined silicone septa and 600 mg sodium chloride was dissolved producing a 20% ionic strength solution.

The samples were prepared by dissolving 50 mg of the topical products into 100 mL of water and vortexing for one minute. Internal standard solution was added to yield a concentration of 0.25 µg/mL and the ionic strengths adjusted to 20%. SPME were performed on these solutions using a 50/30 µm DVB/CAR/PDMS fiber, which was

conditioned according to the manufacturer's instructions prior to analysis. The SPME fiber was exposed directly into the samples and the extraction conditions were optimized for coating selection, exposure time, pH and ionic strength. After sampling, the fiber was withdrawn into the needle and the SPME device was transferred to the IMS for thermal desorption and analysis.

The extracted analytes were desorbed into the IMS drift tube by depressing the plunger on the SPME holder to expose the fiber. The exposed fiber was placed on the desorption tray in the center of the sampling region, and the tray assembly was slid all the way to the injection position, where the desorber rises, sealing the SPME fiber against the heated IMS inlet. Air was drawn through the sampling region at 400 mL/min to transfer the analytes from the fiber into the IMS drift tube for detection (Figure 5.2).

The topical products labeled as Cream A, Cream B, Cream C, Solution A, Lotion A and Ointment A, containing various combinations of the preservatives, were quantitated using the internal standard standard method. HPLC analyses were also performed on the creams, lotion, solution and ointment by dissolving 1g of sample into 10 mL methanol. A standard extraction procedure commonly used in the pharmaceutical industry was followed, which includes vortexing at high speed for 5 minutes, heating in a 90°C water bath for 5 minutes followed by cooling in an ice bath for 5 minutes. The standard solutions were prepared by dissolving 10 mg of the parabens in 50 mL methanol yielding a concentration of 0.2 mg/mL. The samples and standard preparations were filtered through a 0.2 μm filter and subsequently analyzed on an 1100 HPLC (Agilent Technologies, Wilmington, DE). The analyses were performed by injecting 25 μL samples and standards, using a flow rate of 1.5 mL/min of 50:50 acetonitrile/water

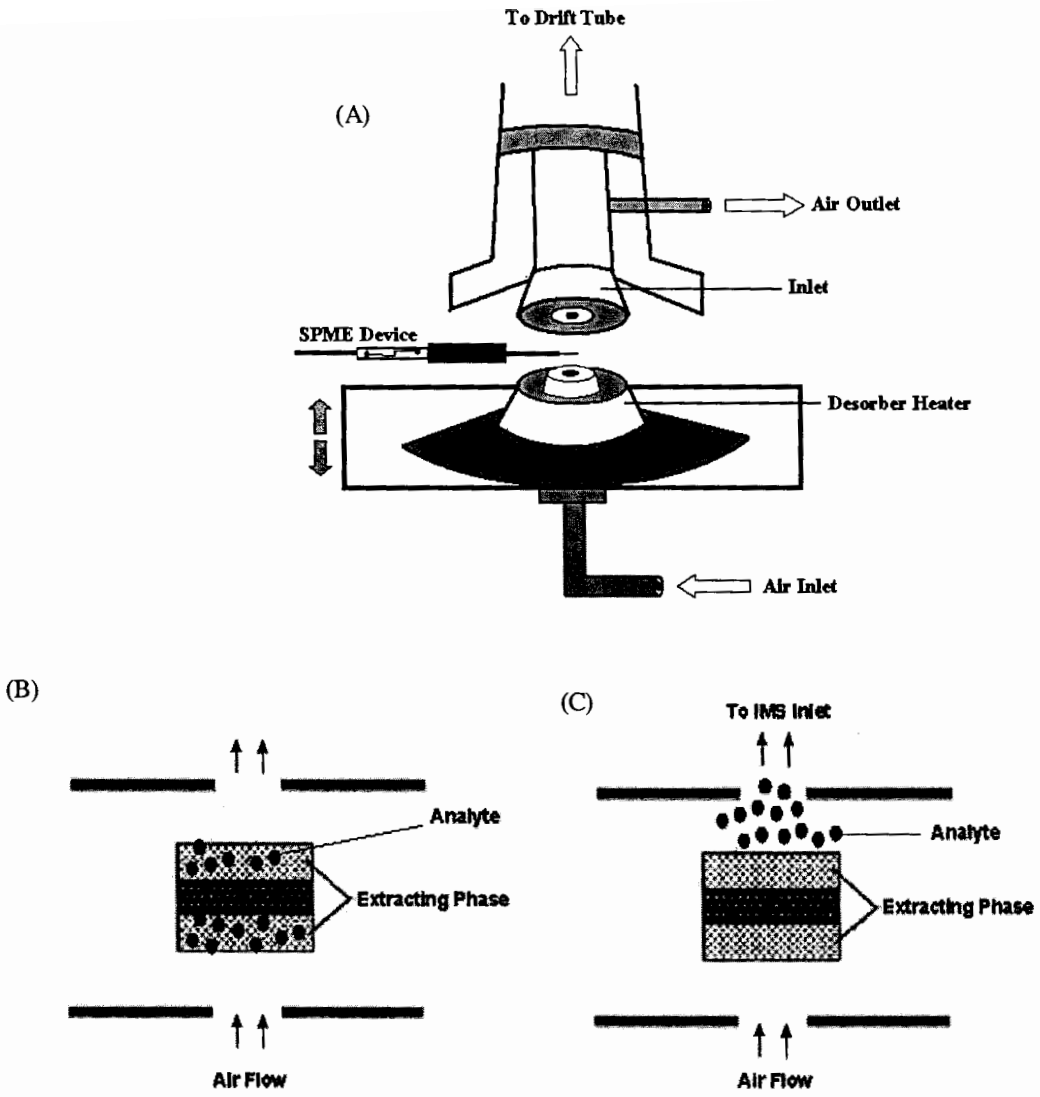


Figure 5.2. . A. Schematic diagram of the SPME–IMS interface, showing the location of the SPME device. B. Dynamics of the desorption process – analytes in the fiber coating. C. Dynamics of the desorption process - analytes evaporating from the coating and entering the flow stream. Note the fiber is oriented 90 degrees differently to the flow than in traditional SPME-GC.

mobile phase and a silica-based C_{18} column with a 5 μm particle size and dimensions of 250 mm x 4.6 mm. Detection was done at 254 nm.

5.4 RESULTS AND DICUSSION

For the production of negative ions, there are three possible reactions that can occur. These include ion transfer, charge transfer, or dissociative charge transfer. In the negative mode, the reactant hexachloroethane is used as the reactant substance. Ionization selectivity is obtained for compounds whose electron affinities are greater than that of the reactant ion through an equilibrium shift that is determined by the relative electron affinities of the reactant and analyte [241].

The advantages of SPME with respect to selectivity and sensitivity render it an ideal sample preparation technique to combine with IMS. Different SPME fibers have different selectivities and sensitivities toward the target analyte. Therefore, different fibers must first be evaluated and then the optimal one selected for further studies.

The choice of an appropriate coating is essential for the SPME method. Five types of fibers were studied: 65 μm polydimethylsiloxane/divinylbenzene (PDMS/ DVB), 65 μm Polyacrylate, 7 μm Polydimethylsiloxane (PDMS), 100 μm Polydimethylsiloxane, and 50/30 μm Divinylbenzene/Carboxen/PDMS (DVB/CAR/PDMS). A 100 mg sample of Cream B, containing methyl paraben and propyl paraben was dissolved in 100 mL water. Ethyl paraben and butyl paraben were spiked into the cream solution to yield a concentration of 1 μg/mL of these two substances. The sample concentration and extraction parameters were kept the same for each study conducted on the five fibers.

Figure 5.3 displays the extraction profiles of the parabens in a cream matrix by different fiber coatings extracted for 15 minutes. This figure shows that the 50/30 DVB/CAR/PDMS fiber exhibit the highest sensitivity to parabens for the 15 minutes duration. The difference in extraction performance amongst the fibers reflects variations in the polarity of the individual fibers and relative sorption affinity for the relatively polar hydroxybenzoates. The 7 μm PDMS fiber exhibited poor sensitivity towards methyl, ethyl and propyl paraben due to its nonpolar and low capacity characteristics. As expected, butyl paraben, which is relatively the most non-polar compound in the group exhibited better responses with the non-polar 100 μm PDMS phases. The polyacrylate fiber produced poor responses for all analytes. The PDMS/DVB fiber, recommended for polar volatiles, exhibited a higher sensitivity for the analytes of interest in comparison with the polyacrylate fiber. Compared to the nonpolar liquid PDMS and the polyacrylate coatings, methyl, ethyl and propyl paraben sensitivities could be increased considerably when the DVB/CAR/PDMS fiber is used. The DVB/CAR/PDMS fiber is generally recommended for the adsorption of relatively polar analytes and hence exhibited good sensitivity for parabens in comparison with the other fibers. The relative polarities of the four parabens are also reflected by the dramatic increase of methyl paraben and ethyl paraben responses with the more nonpolar DVB/CAR/PDMS fiber. For all further studies, the 50/30 μm DVB/CAR/PDMS fiber was chosen and the remaining fibers were not investigated further.

Since SPME is an equilibrium extraction technique, the maximum amount of parabens extracted by the fiber under a given set of conditions is determined by the time to reach adsorption equilibrium. The equilibrium time is reached when a further increase

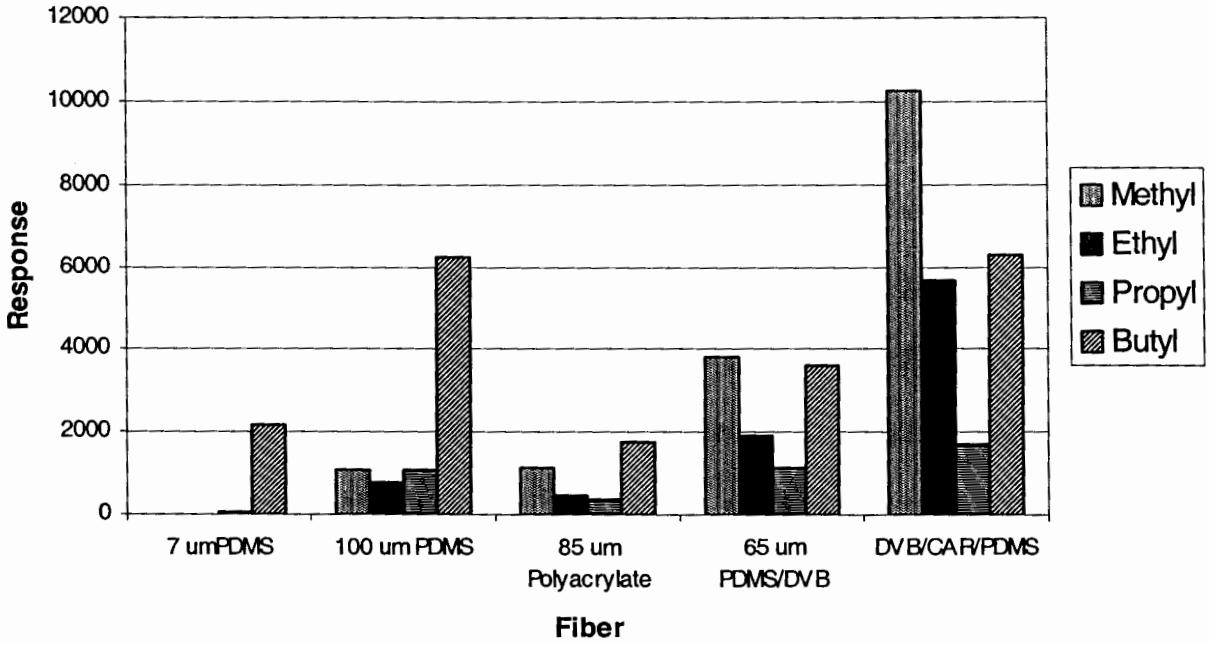


Figure 5.3. Extraction profile of parabens in Cream B, sampled directly by five SPME fibers

of the extraction time does not result in a significant increase in detector response. The efficiency of parabens extraction by SPME was investigated using an extraction recovery-time curve for samples. The study was done by comparing the response of known concentrations of parabens as a function of contact time with the DVB/CAR/PDMS fiber. Six replicate samples, prepared as described above, were extracted at 5, 10, 15, 30, 45, and 60 minutes. As seen in Figure 5.4, the amount of parabens adsorbed to the fiber leveled off after approximately 15 minutes extraction time. Sample extraction time of 15 minutes was therefore chosen to achieve maximum sensitivity without unduly extending the analysis time.

The effect of pH on the SPME extraction of parabens was studied by adjusting the pH of the samples with 0.1 M HCl and 0.5 M NaOH. The pH of the Cream B sample is about 6, and the adjustments were made to pH 4, 5, 7, 8, and 9, followed by extraction with the DVB/CAR/PDMS fiber. As seen in Figure 5.5, the effect of the pH on the sorption of the parabens showed unappreciable changes in the amount adsorbed when the pH was varied from 4 to 8. At pHs higher than 8, the responses decreased noticeably. Therefore, it was not necessary to make any pH adjustments for all further analyses.

Ionic strength can be an important factor in SPME extraction and studies were done by comparing the responses of the parabens as a function of sodium chloride concentration. The responses of parabens in the cream matrix were investigated by performing extraction studies on samples where the ionic strengths of the solutions were adjusted with sodium chloride. Extractions were performed on 3 mL aliquots of spiked Cream B sample with 0%, 1%, 5%, 10%, 20% and 30% of sodium chloride added.

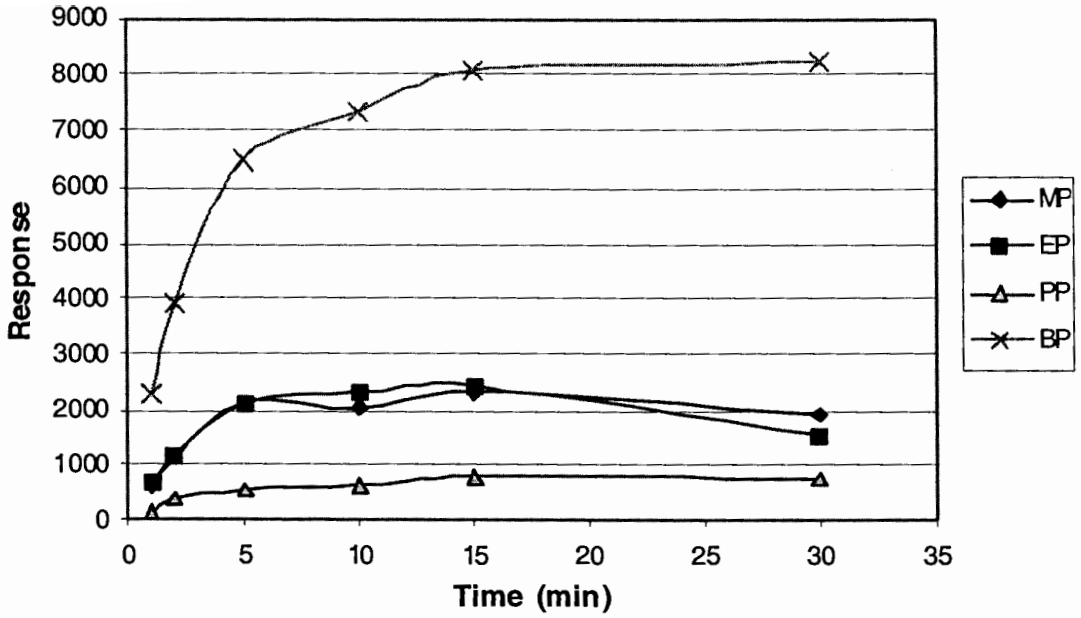


Figure 5.4. Plot of MP, EP, PP and BP responses as a function of sample extraction time by SPME

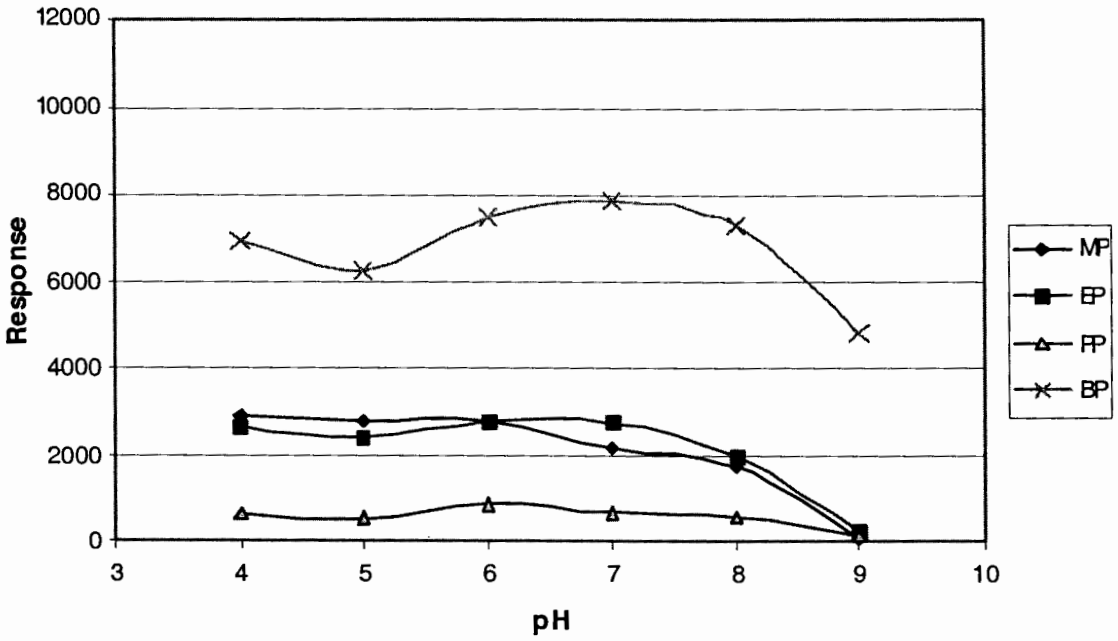


Figure 5.5 Effects of pH on the extraction of MP, EP, PP and BP in Cream B using a DVB/CAR/PDMS fiber

Generally an increase in the ionic strength of the sample will improve the sensitivity of some analytes by increasing the distribution coefficient, which leads to greater sensitivity. This phenomenon is usually more apparent with polar analytes. As seen in Figure 5.6, the peak response of MP and EP increased significantly as the ionic strength increased. There was also a small increase in response for PP as the ionic strength approaches 20%. The peak response of BP increases initially at 1%, but starts to decrease as the salt concentration increases further. Sample ionic strength of 20% was chosen as a compromise to achieve adequate extraction efficiency for all of the compounds.

The amount of analyte compounds adsorbed on the SPME fiber may be dependent on the sample volume. Therefore, studies were performed to determine the optimum sample volume for parabens extraction. SPME were performed on 0.5 μg/mL standard with volumes at 1 mL, 3 mL, 5 mL and 10 mL. Figure 5.7 shows that no noticeable increase in the amount extracted were observed for four analytes as the volume increased from 1 mL to 10 mL. A 3 mL extraction volume was chosen for all analysis.

A plasmagram of a standard solution spiked with MP, EP, PP, BP and internal standard is shown in Figure 5.8. The plasmagram shows sharp Gaussian-shaped peaks for all analytes where baseline separation for the five parabens were achieved in less than 18 ms. The plasmagram is indicative of little or no fragmentation of the analyte ions, formation of ion clusters or decomposition reactions in the drift tube. The peaks present from 7 ms to 12 ms, also present in the blank injections, are produced mainly from the chloride reactant ions and their fragments. A three-dimensional plasmagram of a standard solution containing MP, EP, PP, BP and benzyl paraben prepared is shown in Figure 5.9.

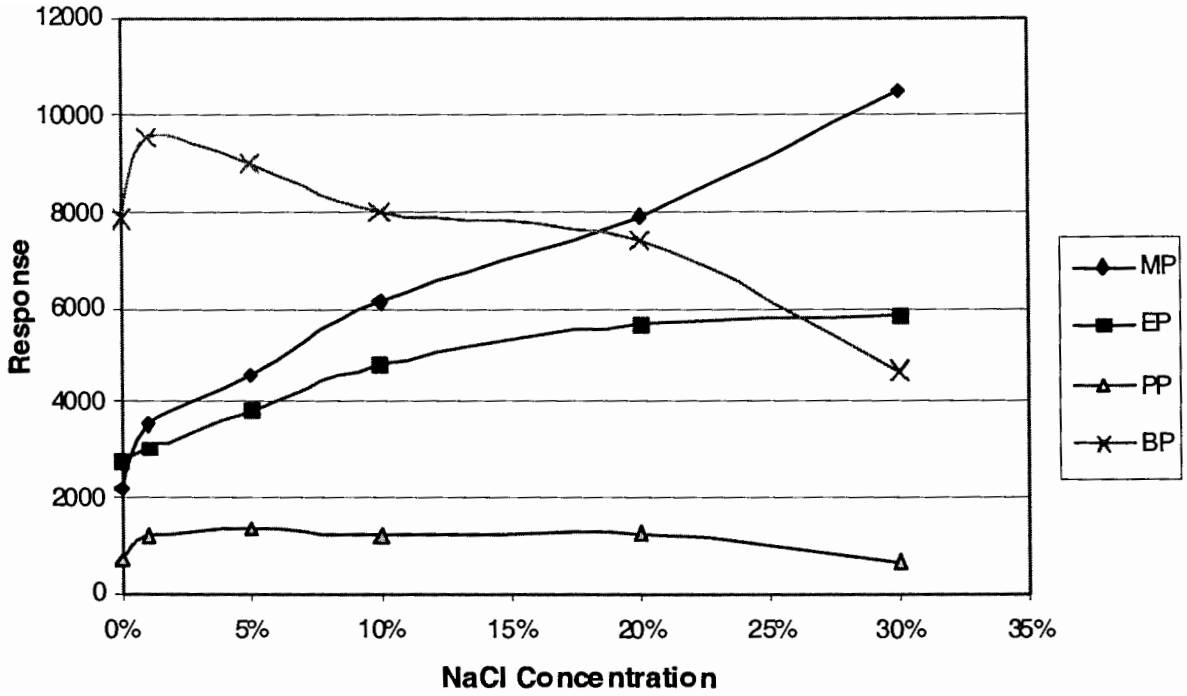


Figure 5.6. Effect of MP, EP, PP and BP responses as a function of sample ionic strength

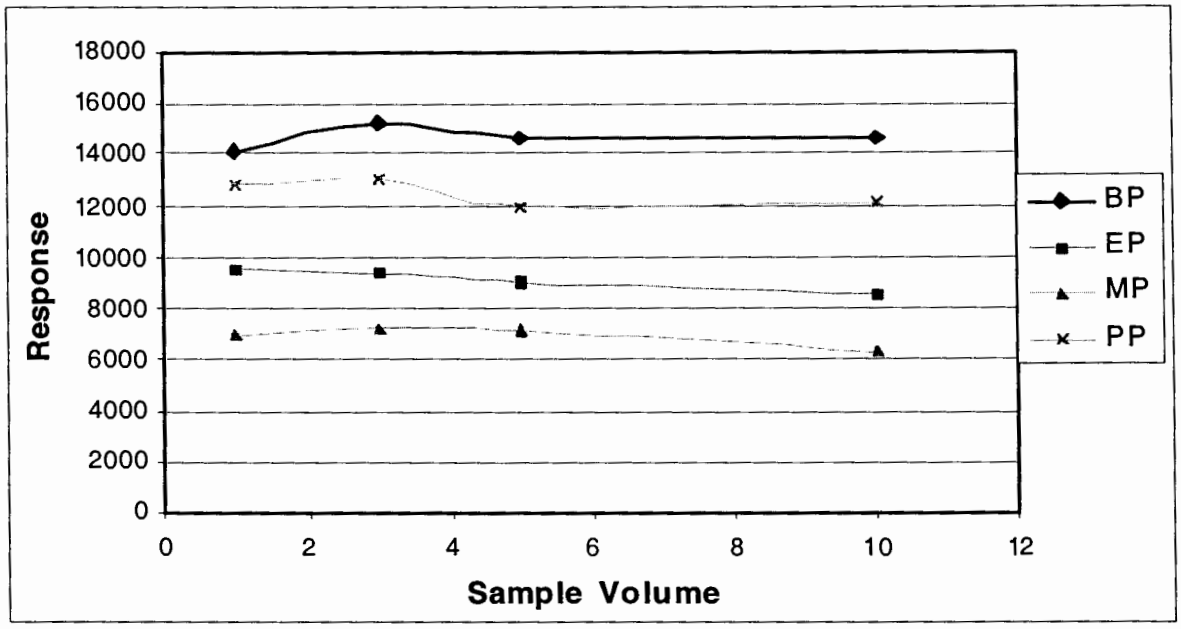


Figure 5.7. Effect of MP, EP, PP and BP responses as a function of sample volume.

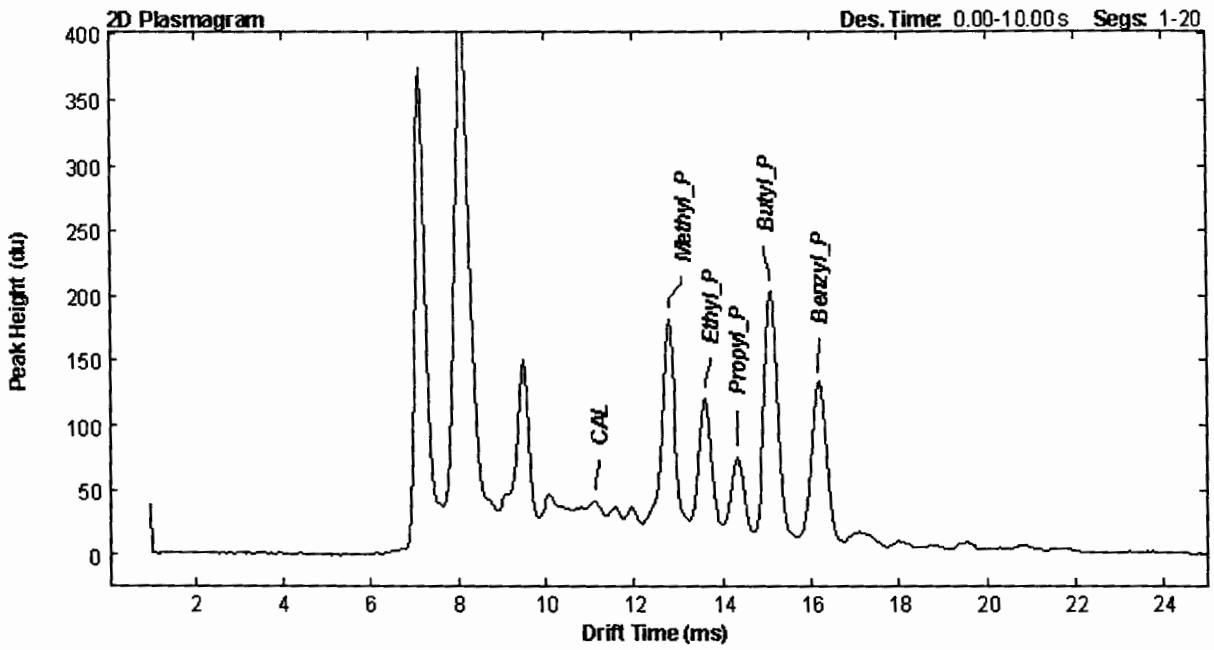


Figure 5.8. A plasmagram of a standard solution containing MP, EP, PP, BP and benzyl paraben prepared in water

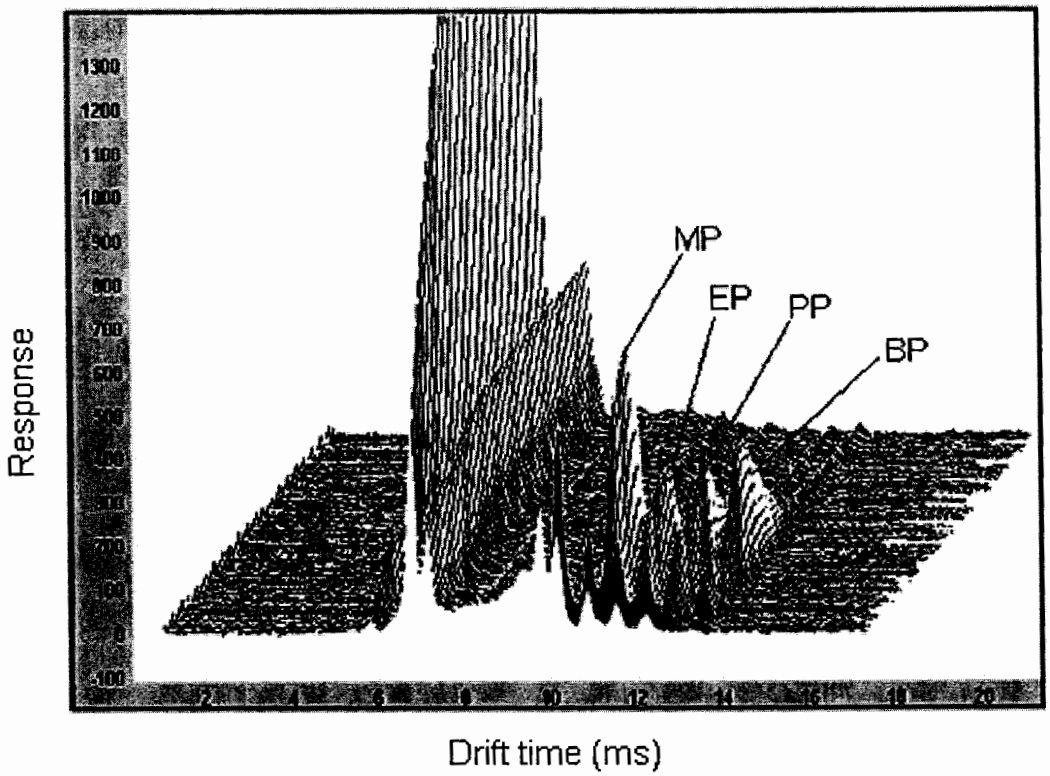


Figure 5.9. A three-dimensional plasmagram of a standard solution containing MP, EP, PP, BP and benzyl paraben prepared in water

The separation efficiency of the SPME-IMS method may be measured in terms of peak-to-peak resolution, R_{pp}, defined on the basis of separation of pairs of adjacent peaks, as in chromatography.

$$R_{pp} = 2\left(\frac{t_{d2} - t_{d1}}{\omega_{b1} + \omega_{b2}}\right) \qquad \text{Eq. 5.1}$$

The resolutions were calculated using Equation 5.1, where t_{d2} and t_{d1} are the drift times of the two adjacent peaks, and w_{b1} and w_{b2} are their respective widths at the base. The resolution values calculated for the parabens were greater than or equal to one, indicative of very good separation for an IMS method. This separation power is interesting considering that the four parabens of interest differ by just a methyl group. The separation efficiency of IMS, also referred to as the theoretical plates (N), is similar to that normally used in chromatography, and is a function of the drift tube voltage, temperature, number of charges on the ion, initial pulse width, length of the drift tube and the mobility of the ion of interest [242]. The theoretical plates were calculated for the parabens, using Equation 5.2, where w_h is the peak width at half the maximum height.

$$N = 5.55\left(\frac{t_d}{\omega_h}\right)^2 \qquad \text{Eq. 5.2}$$

The results calculated for the five analytes determined to be greater than ten thousand theoretical plates, are summarized in Table 5-1.

Compound	Drift time (ms)	Reduced Mobility $cm^2V^{-1}s^{-1}$	Resolution	Theoretical Plates
Methyl paraben	12.746	1.4412	N/A	11163
Ethyl paraben	13.538	1.3569	1.09	11778
Propyl paraben	14.295	1.285	1.04	13023
Butyl paraben	15.053	1.2204	1.00	11342
Benzyl paraben	16.140	1.1382	1.31	12035

Table 5-1. IMS drift times, reduced mobilities, resolutions and theoretical plates of paraben analysis

As IMS theory predicts, smaller ions have shorter drift times compared to larger ions, because they travel faster through the drift tube. The drift times needed by the ions to reach the detector are proportional to their masses but inversely proportional to their characteristic reduced ion mobilities. Many attempts have been made to demonstrate that the masses of ions in a homologous series may be determined from ion drift time measurements. Most of the mass-mobility investigations were done on the homologous series of primary and tertiary aliphatic and aromatic amines. Good correlation have been obtained for mass-mobility measurements using a rigid sphere model involving parameters such as ion interaction potential, ion collision cross section and various mathematical correction factors [243,244].

A plot of the K_o vs. molecular weight for the four parabens of interest is shown in Figure 5.10, and exhibits good linear correlation with an r^2 value of 0.996. This mass-mobility correlation observed without applying any of the traditional model calculations indicates that the benzoate ions produced most likely do not form clusters and are probably molecular ions of the parabens. This correlation can be a very useful tool in estimating molecular weight of unknown ions based on their drift times in IMS. Deviations from the linear curve would likely be observed if additional homologues of the parabens were tested.

The linearity of the method was investigated by determining calibration curves for the four parabens over the concentrations of 0.05 μg/mL to 1.0 μg/mL. The line of best fit for the relationship between the peak response and the concentration of each analyte in the standard solution was determined by linear regression.

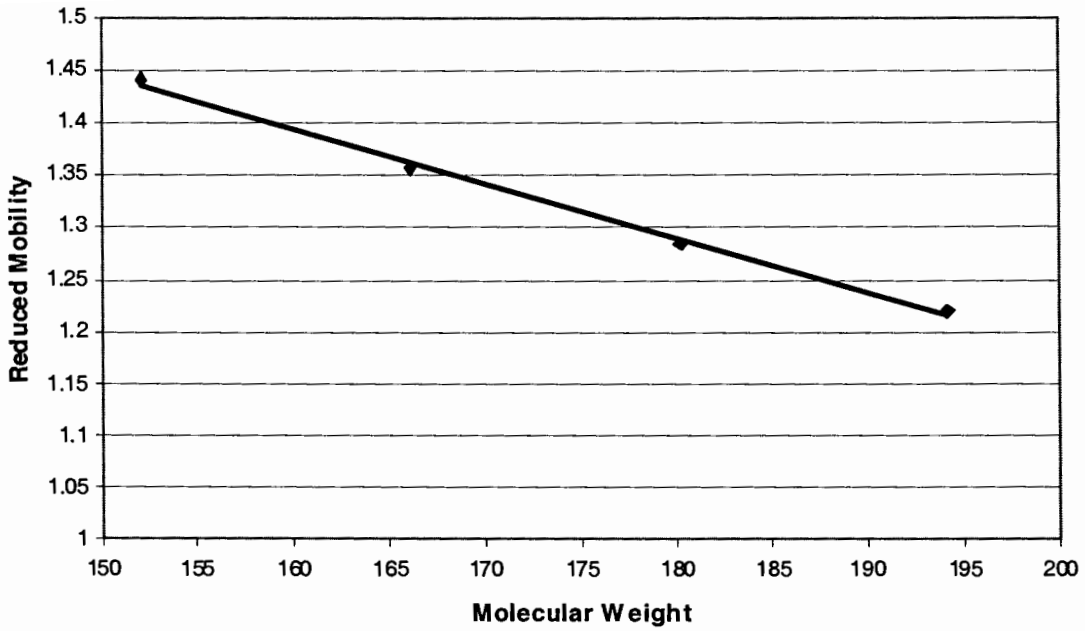


Figure 5.10. A plot of reduced mobilities vs. molecular weight for the MP, EP, PP and BP ions. The mass-mobility correlation exhibits an r^2 of 0.996 for the parabens.

A linear range of three orders of magnitude was obtained for the four parabens. Calibration curves for MP, EP, PP and BP are shown in Figure 5.11, and the results are presented in Table 5-2, together with the results obtained for method precision and detection limits.

The reproducibility of the method with 0.5 μg/mL standards were evaluated and found to have relative standard deviations (N=5) of less than 8%. The results are shown in Table 5-2. The relative standard deviations (N=5) for drift times were 0.5% for all analytes, indicating good repeatability, which is critical for analyte identification purposes in IMS.

The method was applied to various types of commercial topical pharmaceutical formulations. The samples designated as Cream A, Cream B, Cream C, Solution A, Lotion A and Ointment A containing various combinations of parabens were tested by the SPME-IMS method using both external standards and an internal for calibration.

No agitation of the samples was done during extraction because agitation facilitated the extraction of matrix components present in some samples, resulting in ion competition and paraben suppression. The equilibrium time of fifteen minutes, without agitation, was considered practical for routine applications. For the duration of the analyses in this study, the SPME fiber was first exposed onto the IMS injector port as a blank run before the next injection to ensure that the fiber was clean, as well as to avoid any carryover. Since the fiber desorption time was set at 30 seconds, it is feasible to run blanks between analyses.

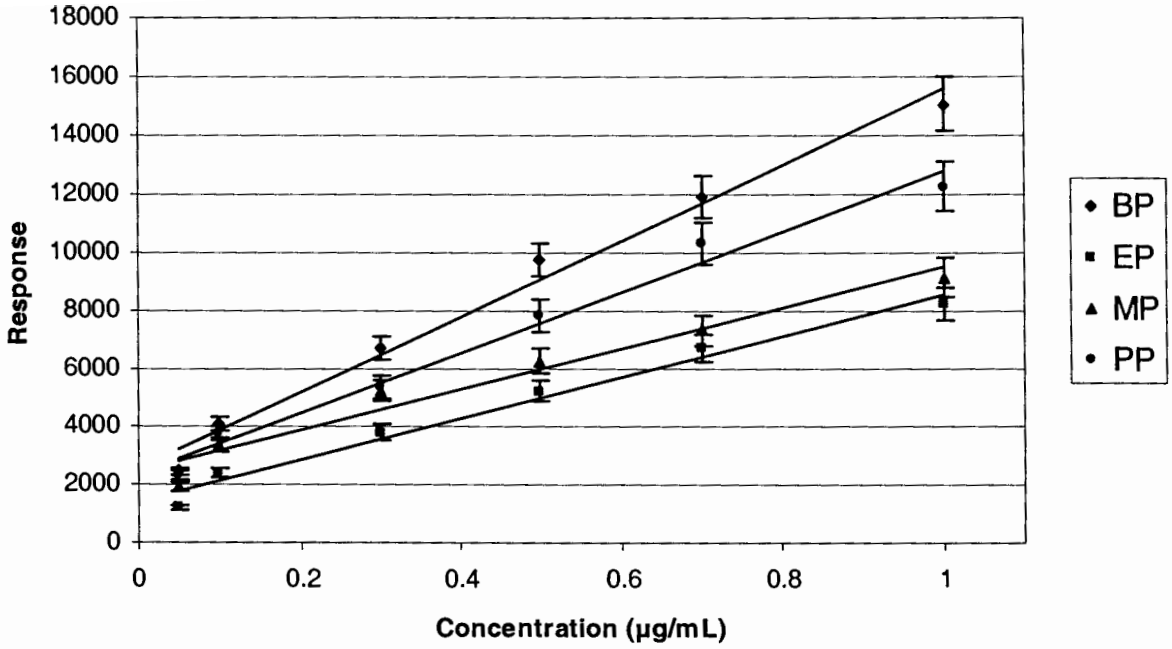


Figure 5.11. Calibration curves for MP, EP, PP and BP at concentrations from 0.05 µg/mL to 1.0 µg/mL by SPME-IMS

	Correlation Coefficient	Precision (% RSD)	Limit of Detection (ng/mL)
Methyl paraben	0.988	7.1	10
Ethyl paraben	0.990	5.8	10
Propyl paraben	0.991	4.3	5
Butyl paraben	0.993	5.7	5

Table 5-2. SPME-IMS method summary showing correlation coefficient, precision and limits of detection for MP, EP, PP and BP.

HPLC is the most common technique that has been validated and used for routine analysis of these preservatives in pharmaceutical topical formulations, and therefore, for comparison purposes, the six samples were tested by this technique to substantiate the results obtained by SPME-IMS. The results for the HPLC method were calculated on a w/w basis and shown in Table 5-3, and Table 5-4 along with the SPME-IMS data.

Although internal standard is the preferred technique for quantitation in SPME, especially for complex matrices, the performance of an external standard calibration was investigated. Standard solutions were prepared by spiking analytes in water and extracted by SPME. Duplicate samples were tested and the mean results calculated in mg/g are presented in Table 5-3.

The SPME-IMS results obtained using external standards show significant differences for the majority of the determinations when compared to the HPLC results. The percent difference ranges from 2% BP in Ointment A to as high as 57% PP in Cream C. The large discrepancies for some determinations indicate that the distribution coefficients of the samples and standards are different for analytes. Therefore different amounts of analyte adsorbs into the polymer phase of the fiber in the clean standard solution compared to the sample solution due to the complexity of the formulations investigated. When sample matrices are simple, such as air or drinking water, the distribution constants are very similar in the standards and samples and therefore an external standard calibration method would be appropriate. For the quantitation of analytes in complex samples, a calibration method such as standard additions or the use of an internal standard is more effective.

Sample	Preservatives	Quantitation by HPLC (mg/g)	Quantitation by SPME/IMS (mg/g)	Percent Difference
Cream A	Methyl paraben	0.98	0.94	4.1
	Propyl paraben	0.27	0.23	14.8
Cream B	Methyl paraben	1.98	2.37	19.7
	Propyl paraben	0.21	0.26	23.8
Cream C	Methyl paraben	1.92	2.16	12.5
	Ethyl paraben	0.41	0.32	21.9
	Propyl paraben	0.23	0.10	56.5
	Butyl paraben	0.45	0.40	11.1
Lotion A	Methyl paraben	1.42	1.29	9.2
	Propyl paraben	0.21	0.11	47.6
Solution A	Methyl paraben	1.65	1.41	17.0
Ointment A	Methylparaben	0.16	0.14	12.5
	Butylparaben	1.48	1.45	2.0

Table 5-3. Contents of MP, EP, PP and BP determined in commercial topical formulations determined by SPME-IMS, quantitated by external standards. Results are compared to HPLC data.

The six products containing various combinations of parabens were also tested using benzyl paraben as an internal standard. Duplicate samples were tested and the mean results calculated in mg/g are presented in Table 5-4. The results for preservatives quantitated by HPLC and internal standard SPME-IMS show good comparison between the two methods. The percent difference between the SPME-IMS and HPLC methods ranges from 1.4% methyl paraben in Lotion A to 14.3 % propylparaben in Ointment A. As seen in Figure 5.12, the difference obtained between the two methods for the products tested is not significant for most determinations when the uncertainties for each method are taken into consideration.

Due to proton affinities and ion competition effects produced by substances present in a sample, when mixtures are injected directly into IMS, analytes can be completely missing or largely suppressed from plasmagrams until their concentrations become larger than the other compounds present in the sample. Historically, this limitation has been problematic when quantitative determination is necessary for complex samples. Therefore, it was remarkable that significant discrepancies were not seen in the SPME-IMS results for parabens since these preservatives are a very small percentage of the total amount of active and excipient substances present in the samples.

With the exception of MP in Cream C, generally, the larger differences between the two methods occur where the parabens concentrations are less than 0.5 mg/g Additional optimization of the SPME extraction method for each matrix type can further minimize the discrepancies observed for some determinations. For samples containing higher levels of interfering compounds, additional extraction parameters can be adjusted to achieve more efficient paraben recoveries.

Sample	Preservatives	Quantitation by HPLC (mg/g)	Quantitation by SPME/IMS (mg/g)	Percent Difference
Cream A	Methyl paraben	0.98	1.00	2.0
	Propyl paraben	0.27	0.24	11.1
Cream B	Methyl paraben	1.98	1.90	4.0
	Propyl paraben	0.21	0.19	9.5
Cream C	Methyl paraben	1.92	2.17	13.0
	Ethyl paraben	0.41	0.37	9.8
	Propyl paraben	0.23	0.20	13.0
	Butyl paraben	0.45	0.50	11.1
Lotion A	Methyl paraben	1.42	1.44	1.4
	Propyl paraben	0.21	0.18	14.3
Solution A	Methyl paraben	1.65	1.68	1.8
Ointment A	Methyl paraben	0.16	0.18	12.5
	Butyl paraben	1.48	1.41	4.7

Table 5-4. Contents of MP, EP, PP and BP determined in commercial topical formulations determined by SPME-IMS quantitated using benzyl paraben as an internal standard. Results are compared with to HPLC data.

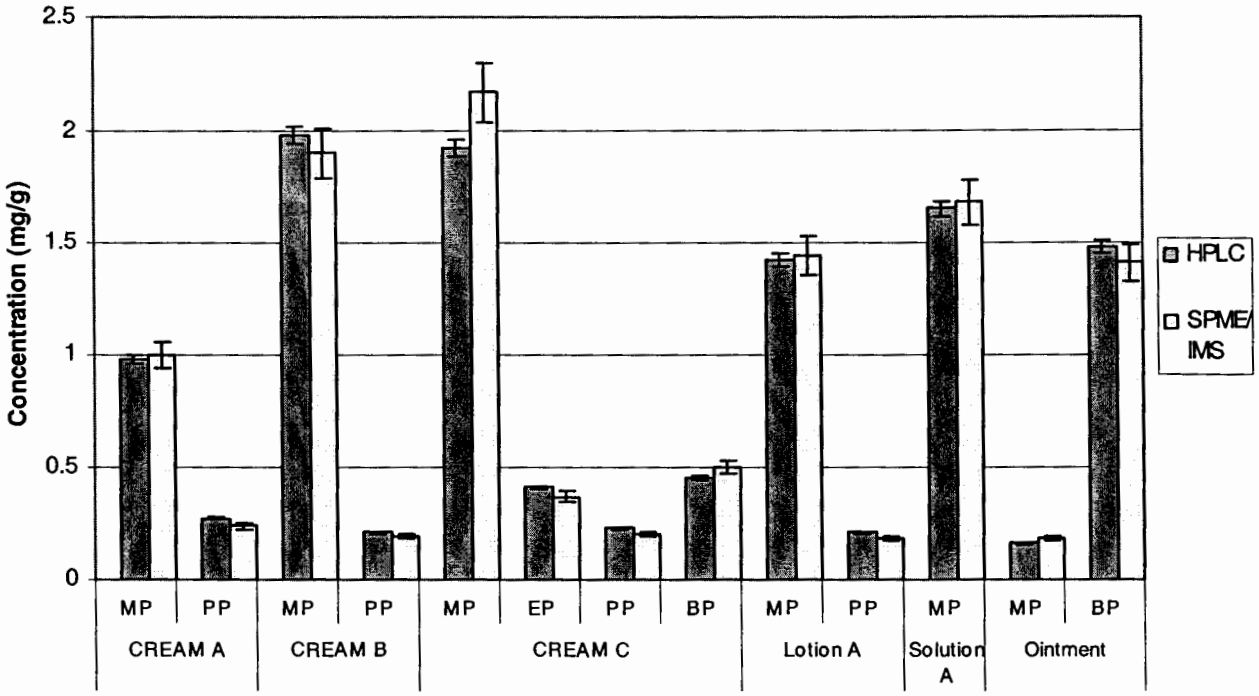


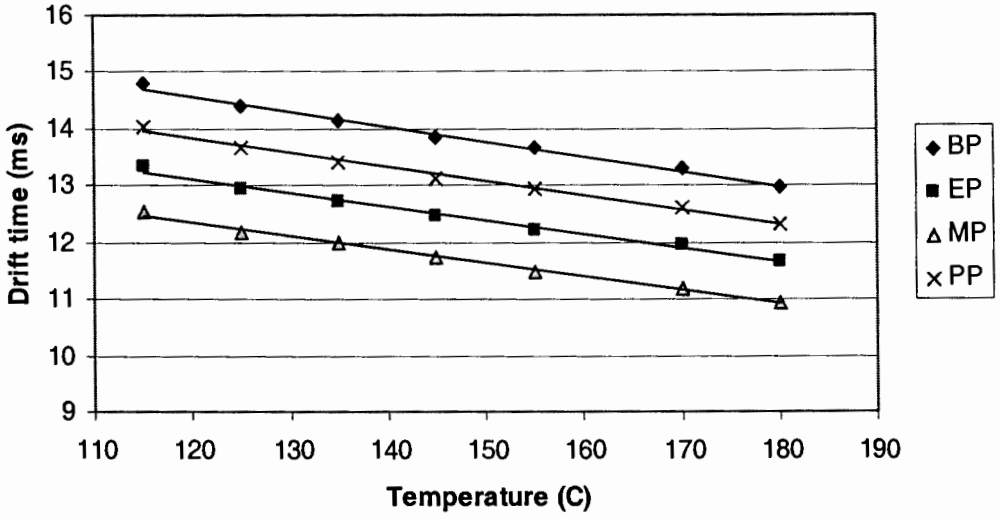
Figure 5.12. Comparison of the results obtained for HPLC and internal standard SPME-IMS. The uncertainties of each method are shown with the preservatives for the six products tested.

Quantitation by both SPME and IMS has been shown to be highly dependent on the matrix composition. Since ion suppression has traditionally been a major limitation in the application of IMS to detect and quantitate analytes in complex matrices, the relative composition of the liquid matrix should be considered carefully when the data obtained using these methodologies are used. It was expected that in the real samples tested, differences in proton affinities, vapor pressures, and relative composition would have resulted in preferential ionization of components and suppression of the paraben peaks in the mobility spectrum. However, the amount of parabens recovered were not significantly affected due to the selectivity of the SPME extraction procedure and also the elimination of positive ions, obtained by running the IMS in the negative mode. The minimal matrix interferences and lack of ion suppression are reflected in the closeness of the results obtained by the chromatographic method.

The great advantage of the SPME-IMS method is the ability to determine preservatives in the presence of other components in the sample with acceptable confidence without the need to perform chromatographic separation, such that the time and cost of analysis per sample is significantly reduced. The closeness in quantitative results obtained from the two orthogonal methods strongly confirms the applicability of the proposed SPME-IMS method as a reliable technique that can be used for routine analysis of the parabens.

The influence of drift tube temperature from 115°C to 180°C on the parabens drift times is illustrated in Figure 5.13A. Drift time and mobility (for a given temperature) are related as defined in Equation 5.3, where $v_d(T)$ is the drift velocity in cm/s, $t_d(T)$ is the drift time in s, K(T) is the mobility in $cm^2/V*s$, L is the drift tube length in cm and E is

A



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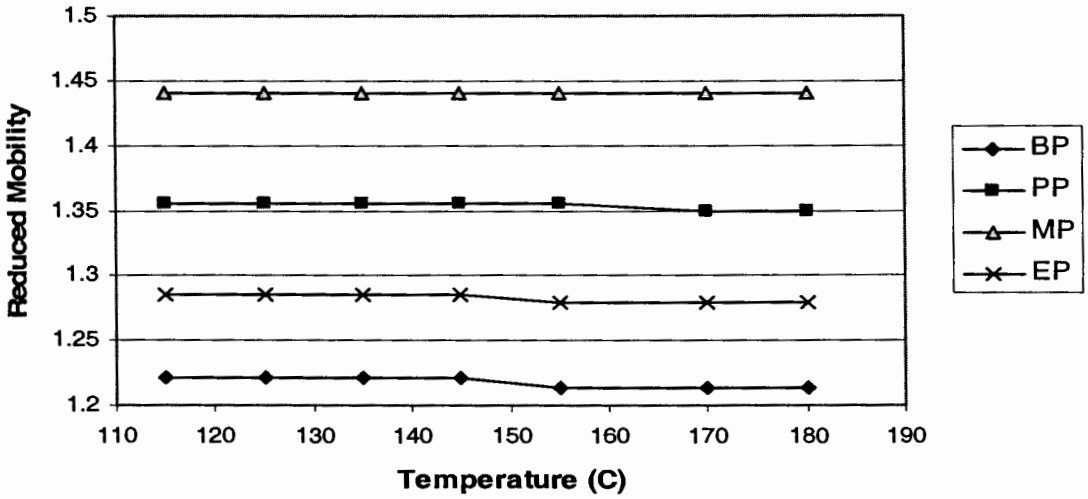


Figure 5.13. Parabens drift time relationship with IMS drift tube temperature. Temperature increases from 115°C to 180°C results in a faster ion velocities and shorter drift times. The drift times are shown in (A) and the calculated reduced mobilities in (B). The reduced mobility (K_0) values are not significantly affected.

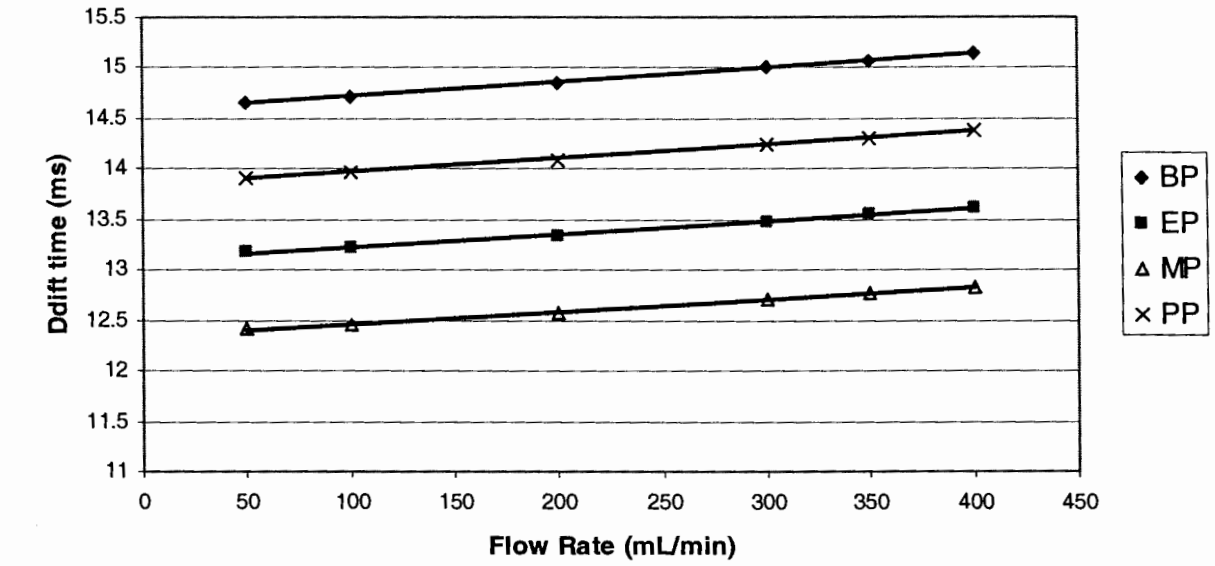
the electric field of the drift region in V/s.

$$v_d(T) = K(T) * E = L/t_d(T) \qquad \text{Eq. 5.3}$$

The drift times for the four parabens decrease proportionally as the temperature increases from 115 °C to 180°C, indicating that the ion velocities are higher at elevated temperatures. For the purpose of standardization, the drift times of ions are typically expressed as reduced mobilities, K_0:

$$K_0 = K * (273/T) * (P/760) \qquad \text{Eq. 5.4}$$

Where T is the temperature in Kelvin and P is the pressure in torr. The mobility of an ion in the drift tube depends on its average collisional cross section, which in turn depends on ion mass, shape, size and charge distribution. Figure 5.13B shows that the reduced mobilities (K_0) of the parabens remain almost constant as the temperature is increased. Although a very small decrease in K_0 is observed after 150°C, the ions produced apparently do not change characteristics in terms mass, shape or size. The relatively flat responses for K_0 indicate that the paraben ions produced are very robust and do not fragment, or change ion-ion and ion-water clusters as the temperature is increased to 180°C. The influence of drift tube gas flow from 50 to 400 mL/min on the parabens drift times is illustrated in Figure 5.14A. As expected the drift times for the parabens increase



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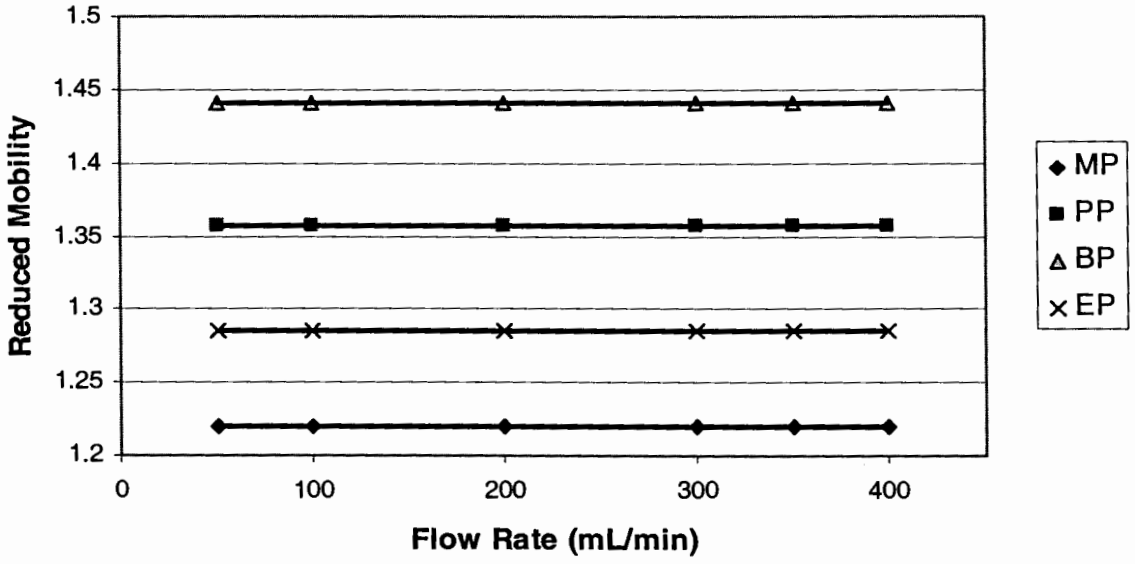


B

Figure 5.14. Parabens drift time relationship with drift tube gas flow. Flow rate increases from 50 mL/min to 400 mL/min results in a slower ion velocities and slightly longer drift times. The experimental drift times are shown in (A). Calculated reduced mobilities of the paraben ions shown in (B) are unaffected.

as the flow rate goes up. This linear correlation is observed because gas and the paraben ions flow in opposite directions in the drift tube. As the flow rate increases the ions are more strongly impeded resulting in longer t_d for each analyte. Higher flow rates can sometimes be used to improve separation. However, in these studies conducted where the instrument drift tube is approximately 7 cm, and the largest ion reaches the detector in less than 18 ms, the effect of increasing the flow rate by a few hundred mL/min has minimal impact on the paraben drift times and resolution.

Figure 5.14B shows the relationship between the carrier gas flow rate and the parabens reduced mobility. As expected, varying the flow rates do not have an impact on the reduced mobility, indicating that the parabens ion shape, sizes and charge distribution are unchanged.

CONCLUSION

This research project describes the successful development of an SPME-IMS method as a new technique for the determination of methylparaben, ethylparaben, propylparaben and butylparaben in pharmaceutical cream, lotion, solution and ointment samples. The method does not require elaborate sample preparation or chromatographic separation and provides a straightforward, fast, sensitive and reliable method for detecting and quantifying the preservatives in complex pharmaceutical matrices. The IMS also operates at atmospheric pressure and does not require carrier gas or mobile phase.

The method reproducibility of less than 8%, linear range of 3 orders of magnitude and sensitivities of less than 10 ng/mL render the SPME-MS method very attractive and promising for routine applications. The excellent separation of the four analytes and internal standard with resolution ≥ 1 and the theoretical plates $>10{,}000$ for all parabens in less than 18 ms is also remarkable for an IMS method. The constant reduced mobility and the preservation of the ion separation at various temperature and flow rates also implies that the method is very robust.

Employing the internal standard, benzyl paraben provided much better reproducibility and recoveries compared to an external standard. The quantitation of preservatives in the real samples using HPLC and SPME-IMS indicate good agreement between the methods with differences in paraben recoveries ranging from 2% to 14.3%. Some of the larger discrepancies between the two methods are observed in samples that contain high levels of interfering compounds with respect to parabens, which result in ion interferences. The main advantages of the SPME-IMS method over HPLC include the reduction of labor-intensive extraction steps, elimination of solvent consumption in extraction and mobile phase preparation, and shortened analysis time from hours to minutes. With appropriate validation for each matrix type, the method can be easily applied to quality control, on site manufacturing in-process testing and field analysis. The good selectivity, separation capability, limit of detection, linear range, reproducibility, and recovery render the SPME-IMS method very attractive and promising for routine analysis.

CHAPTER 6

STIR BAR SORPTIVE EXTRACTION AND THERMAL DESORPTION-ION MOBILITY SPECTROMETRY (SBSE-IMS) FOR THE DETERMINATION OF TNT AND RDX IN WATER SAMPLES

6.1 ABSTRACT

A new technique has been developed for the detection and quantitation of trace analytes using stir bar sorptive extraction (SBSE) interfaced with ion mobility spectrometry (IMS). The sampling technique retains its inherent advantages as a sensitive, straightforward, solventless, and inexpensive extraction method. Additionally, the new SBSE-IMS technique exhibits excellent sensitivity, has on site field analysis capabilities and provides the ability to detect and quantitate analytes that are difficult to accomplish using gas chromatography or high performance liquid chromatography. Due to increased threat of terrorism and the urgent need of various law enforcement agencies and the United States Department of Homeland Security to possess field analysis techniques that are exceptionally sensitive and fast, the explosives 2,4,6- trinitrotoluene (TNT) and hexahydro-l,3,5-trinitro-1,3,5-triazine (RDX) were selected as analytes for the technique's evaluation. The SBSE technique is shown to be an effective on site method for the low-level detection and quantitation of TNT and RDX with good precision, limits of detection and speed of analysis.

6.2 INTRODUCTION

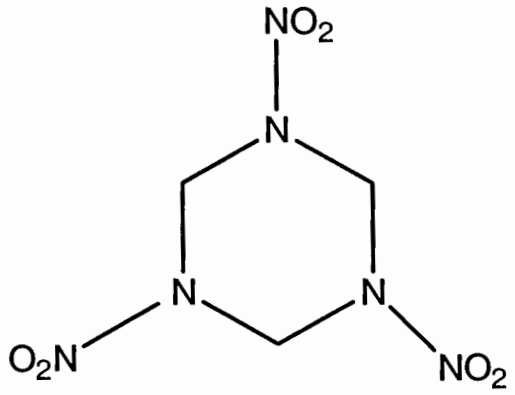
Since its introduction in 1999 by Baltussen et al. stir bar sorptive extraction (SBSE) has been proven to be an effective and sensitive extraction technique for volatile and semivolatile compounds from aqueous samples [245]. The extraction device consists of a magnetic stir bar encased in a thin glass sheath. The outer most portion of the device is a layer of polydimethyl siloxane (PDMS) into which analytes partition. Due to the larger amount of the sorptive phase relative to solid phase microextraction (SPME), a lower phase ratio (volume of water/volume of coating) exists, which increases the recovery of analytes and thereby enhances sensitivity. Although SBSE has been successfully applied for a wide range of analytes from environmental, biological and food samples [246-249], the technique has not been explored for the analysis of trace explosives.

The primary instrumental technique used in tandem with SBSE has been gas chromatography (GC) employing thermal desorption via a specially designed thermal desorption unit (TDU). Due to the larger amount of sorptive phase used in SBSE, the thermal desorption of analytes into the GC is very slow compared to SPME, which necessitates a cold trapping and a re-concentration step prior to column separation. Although solvent desorption, followed by high performance liquid chromatography (HPLC) has been reported in few applications [250,251], the technique is less practical and exhibits lower sensitivity due to the dilution of analytes in the desorbing solvent.

The aim of this study was to develop a technique utilizing the extraction and pre-concentration capabilities of SBSE combined with the speed, sensitivity and portability

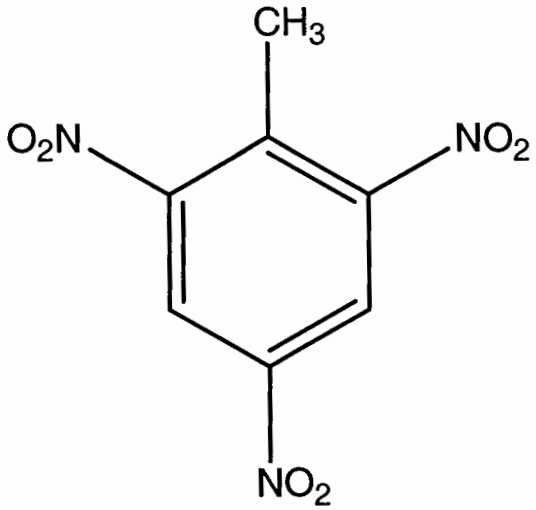
power of ion mobility spectrometry for the determination of explosives in aqueous samples. Although IMS has been extensively employed for the detection of explosives, analyte extraction from environmental matrices continue to be challenging, since explosives are readily soluble in water and greater sensitivity is needed. SBSE serves as an invaluable tool not only to extract analytes from the sample matrix, but also to pre-concentrate and assemble analytes in a form that is amendable for IMS analysis. In SBSE-IMS, the extraction process for the stir bar is similar to that used for GC and HPLC analysis. The major difference between the SBSE-chromatographic techniques and SBSE-IMS is the desorption procedure. The direct desorption of TNT and RDX from the SBSE directly into the IMS using thermal energy along with a high carrier gas flow is shown to be efficient, sensitive and much faster than traditional TDU desorption for GC. The SBSE-IMS technique also possesses the capability to detect and quantitate analytes that are not amendable or difficult to analyze by GC or HPLC, such non-chromophoric, nonvolatile and polar compounds.

With terrorist activities and awareness at an all-time high, the demand for fast, sensitive and on-site explosive detection technology by law enforcement agencies are surging. There is also significant amount of the most common explosives TNT and RDX present in soils and groundwater left behind from mines and military institutions that continue persist and provide a health hazard [252-254]. The new technique of interfacing SBSE to IMS meets the requirement for a portable, fast and sensitive means of detecting and quantitating TNT and RDX from environmental samples. Figure 6.1 shows the structures of the two analytes along with molecular formula and molecular mass information.



RDX

Structural name: 1,3,5-Trinitro-1,3,5-triazinan
Molecular formula: $C_3H_6N_6O_6$
Molecular weight: 222.12



TNT

Structural name: 2,4,6-trinitrotoluene
Molecular formula: $C_7H_5N_3O_6$
Molecular weight: 227.13

Figure 6.1. Structures of RDX and TNT shown with molecular formula and molecular mass information

In this research project the SBSE-IMS technique was explored, developed and reported for the detection and quantitation of TNT and RDX from water. Since SBSE has not previously been examined for these analytes, the extraction method was investigated and optimized. The thermal desorption of analytes from the stir bar has also not been thoroughly examined previously with the TDU for GC analysis. In this study the kinetics of the stir bar desorption using the IMS interface was investigated utilizing the capability of IMS to produce real time snapshots of the thermal desorption process. Temperature, flow rates and desorption times were optimized to prevent carryover. The precision, linearity and detection limits of the new technique are also reported.

6.3 EXPERIMENTAL

6.3.1 Chemicals and materials.

The SBSE-IMS system evaluated for this study is shown in Figure 6.2. The system consists of three parts: (1) the stir bar sorptive device, (2) the interface containing the SBSE device, and (3) the ion mobility spectrometer. For clarity, only the desorber and inlet portion of the IMS are shown in the Figure 6.2. The 1 cm stir bar coated with polydimethysiloxane was obtained from Gerstel (Baltimore, MD). The SBSE device was conditioned according to the manufacturer's specification and several blank desorptions were performed at 280 °C for one-minute intervals to ensure that a stable baseline was obtained on the IMS prior to sample extraction and desorption.

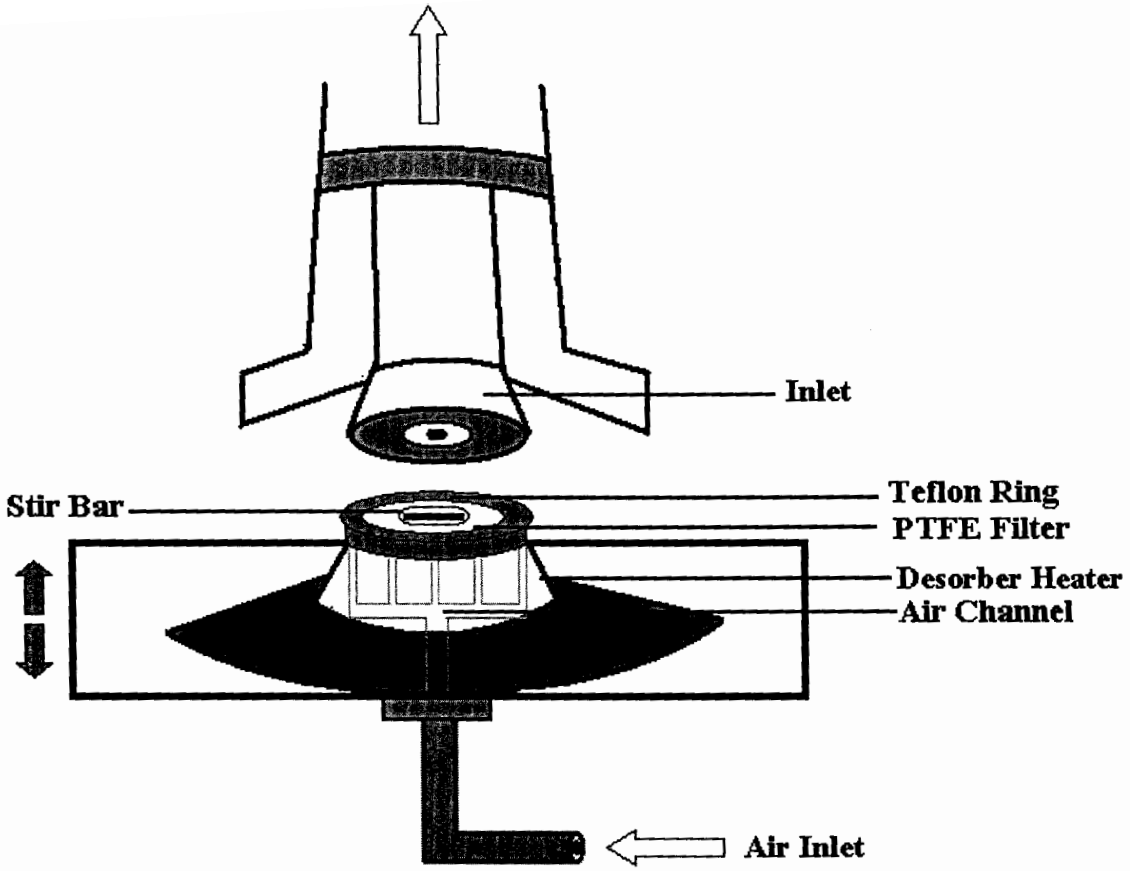


Figure 6.2. The SBSE-IMS interface showing the stir bar present in the desorption compartment along with gas flow patterns

The interface developed for the SBSE-IMS system consisted of: (1) a 2 μm PTFE filter and, (2) a 2 cm diameter x 0.5 cm height Teflon ring. The 2 μm PTFE filter obtained from Whatman (Florham Park, NJ) forms the base of the desorption receptacle, and primarily serves as the support platform for the stir bar during desorption. This filter allows carrier gas to flow through unimpeded to the stir bar. The wall of the desorption receptacle was completed by using a 2 cm diameter x 0.5 cm height Teflon ring that forms a seal between the desorber unit present below and the inlet manifold aligned above. The PTFE filter and the Teflon ring are locked into place on the IMS desorption tray to prevent movement during desorption. The Teflon ring, with the tight seal produced by the vertical movement of the desorber unit, permits the flow of gas through the receptacle containing the stir bar into the IMS inlet The desorber unit and the inlet heats the SBSE desorption chamber from below and above, respectively.

6.3.2 IMS Parameters

The ion mobility spectrometer used in this study was an Ion Scan LS (Smiths, Warren, NJ) programmed in the negative mode, using 4-nitro-benzylnitrile as the calibrant and hexachloroethane as a dopant. Purified air was used as the drift flow gas. The IMS was run with the desorption temperature set at 280 °C, the inlet temperature set at 280 °C, the drift tube temperature at 115 °C and the flow rate set at 400 mL/min. Spectra were collected after a 1 ms delay with a shutter grid width of 0.2 ms. The scan period was set to 30 ms and the desorption time was set at 60 seconds.

TNT and RDX standards were obtained from Supelco (Bellefonte, PA) at a concentration of 1000 µg/mL in acetonitrile. Ultra-pure water used in all experiments was obtained form a Milli-Q unit (Millipore, Bedford, MA) installed in the laboratory.

6.3.3 Methods

Standard stock solutions of TNT and RDX obtained at 1000 µg/mL were diluted in water to produce a working solution containing 5 ng/mL TNT and 50 ng/mL RDX. Aliquots of 10 mL of the working solution were transferred to a 12 mL Erlenmeyer flask. The preconditioned stir bar was then added to the flask and stirred at 1000 rpm on a magnetic stirring plate for 30 minutes. After extraction, the stir bar was then carefully removed from the solution using a stainless steel wire and transferred onto the PTFE filter in the center of the desorption chamber. The tray assembly containing the desorption chamber was slid all the way to the injection position, where the desorber rises, sealing the teflon ring against the heated IMS inlet. Air was drawn through the sampling region at 400 mL/min to transfer the analytes from the stir bar into the IMS drift tube for detection.

6.4 RESULTS AND DISCUSSION

For the evaluation of the SBSE-IMS interface, TNT and RDX were used as the test samples since these are the most common explosives used and found in the environment. RDX has also traditionally been extremely difficult to extract and analyze using classical

extraction and chromatographic techniques due to the polarity and low vapor pressure of the compound. In previous work done on explosives, several publications reported many challenges in detecting RDX from real-world samples [255-258].

The PDMS sorptive coating on the stir bar is non-polar and therefore SBSE has a greater affinity for absorbing non-polar compounds. Not surprisingly, when equal concentrations of the explosive were spiked in water and extracted by SBSE, TNT exhibited a ten times greater response compared to RDX. Because TNT and RDX exhibit similar IMS response factors, determined experimentally by direct injection, it can be concluded that the recovery of TNT with its conjugated double bonds results in an SBSE recovery that is approximately ten fold greater than the more polar RDX. Therefore for simplicity, all studies were performed using samples containing TNT and RDX concentrations at a ratio of one to ten.

The extraction procedure for SBSE-IMS was similar to the SBSE-GC method described by Baltussen et al. However, since SBSE has not been previously attempted for explosives analysis, an extraction time profile study was done to determine the optimum extraction time for these analytes. Samples used in this study consisted 10 ng/mL TNT and 100ng/mL RDX spiked in 10 mL aliquots of water. The extractions were carried out as described above at 5, 20 30, 45 and 60 minutes. The plot of the extraction time profile is shown in Figure 6.3, where equilibrium for both TNT and RDX were achieved at approximately 30 minutes.

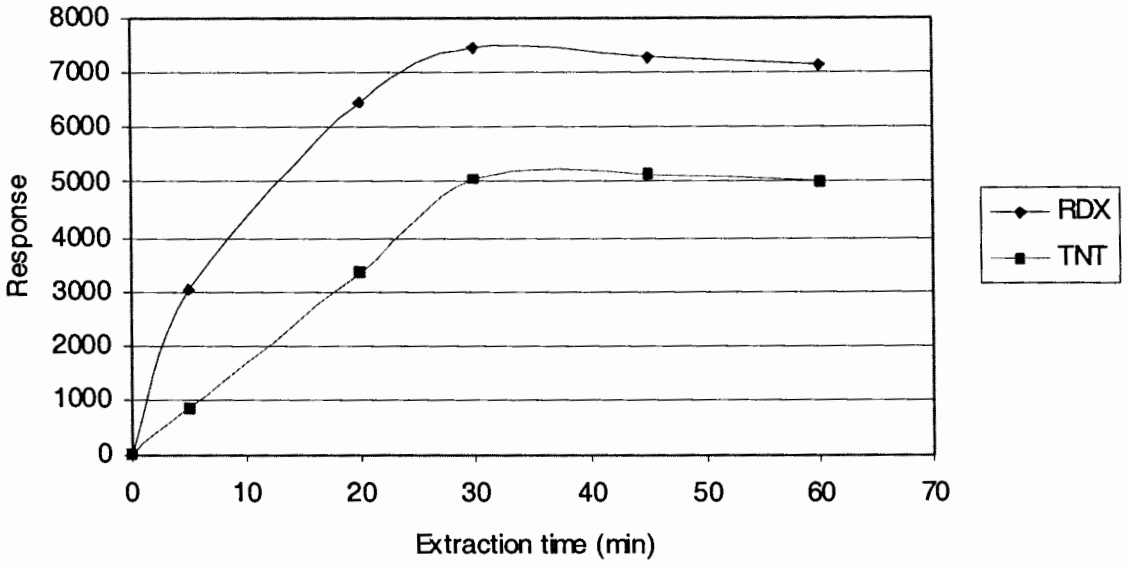


Figure 6.3. Extraction time profile of TNT and RDX by SBSE using a desorption temperature of 280°C, flow rate of 400 mL/min for 60 seconds.

Sample pH and ionic strength adjustments have been reported to improve the extraction efficiency of SBSE. Therefore, both of these parameters were adjusted independently using 0.1 M sodium hydroxide, 0.1 M hydrochloric acid and sodium chloride to determine the effect on explosives recovery. Neither the pH nor the ionic strength adjustments produced any appreciable improvement in the recovery of TNT or RDX. As a result no changes were made to the sample matrix for further studies. The desorption time for the stir bar was varied between 30 and 90 seconds. It was found that peak responses increased up to 60 seconds, but remained fairly constant at higher desorption times. All remaining investigations were performed at 60 seconds stir bar desorption.

Figure 6.4 depicts the plasmagram obtained for TNT and RDX by direct injection and also using the SBSE-interface under the conditions previously described. Good separations of the peaks were obtained for all analyte ions produced. The TNT and RDX peaks obtained by SBSE-IMS are Gaussian-shaped, well defined, and sharp. The plasmagram compares well with little differences in shape or resolution compared to the peaks produced by direct injection. TNT exhibits a drift time of 12.663 ms. RDX produces two peaks with the major one at 13.239 ms and a secondary peak at approximately 13.7 ms. The major peak for RDX at 13.239 was used for all method optimization and quantitation purposes The peaks present in the early portion of the plasmagram between 6ms and 12 ms, which also occur in blank injections, are product ions from the reactant ion, hexachlorethane and also the calibrant 4-nitro-benzylnitrile. In the SBSE desorption of blanks and samples, considerable baseline disturbances were observed in the plasmagrams between 15 and 19 ms. For identification purposes, reduced

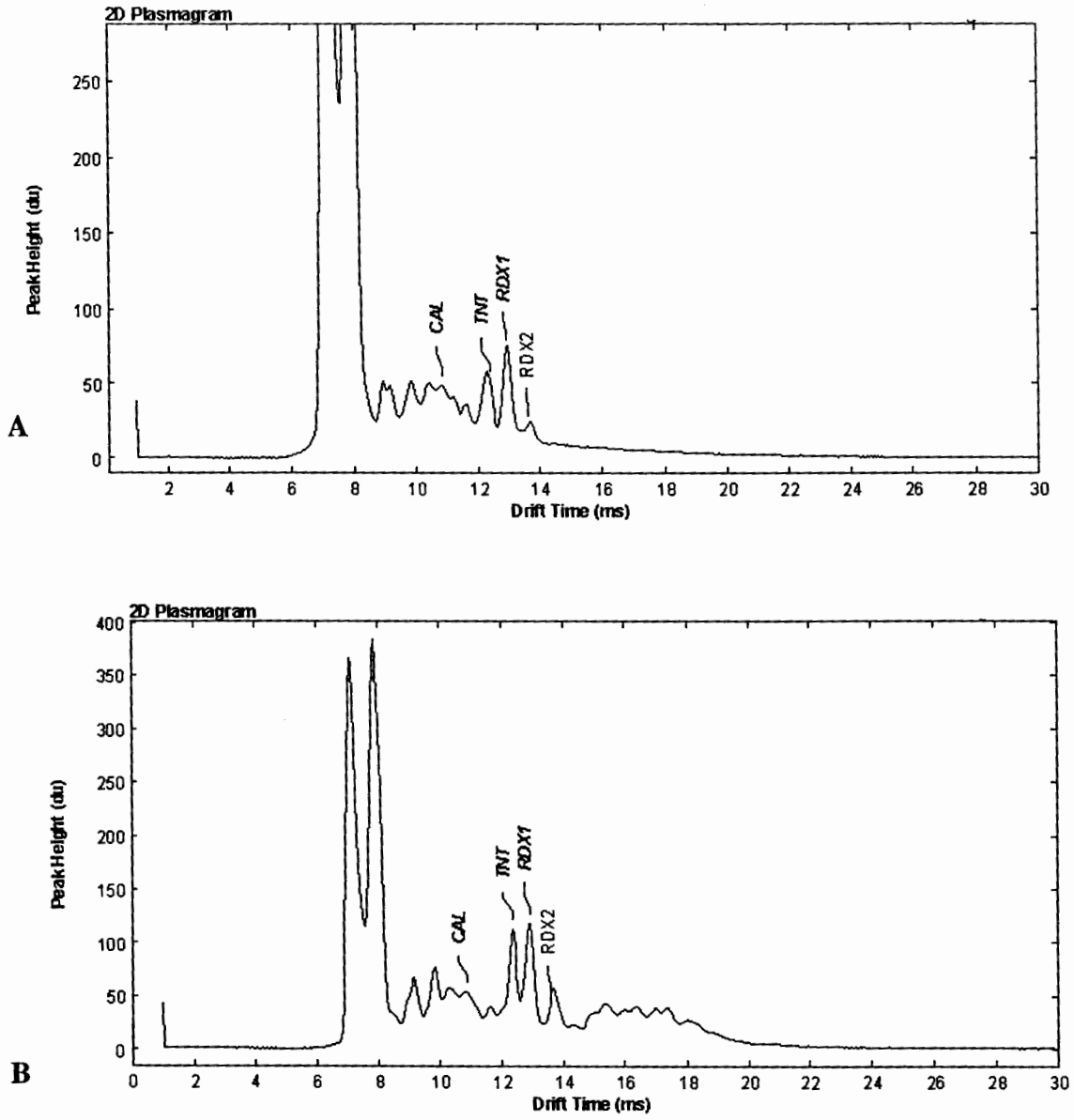


Figure 6.4. Plasmagrams of TNT and RDX acquired by (A) direct injection of analyte solution prepared in acetone and (B) SBSE-IMS of analytes extracted for 30 min and desorbed at 280°C, with a flow rate of 400 mL/min for 60seconds.

mobilities (K_0) are often used instead of drift times to correct for instrumental and environmental variations. The K_0 values are calculated in negative ion mode using 4-nitro-benzylnitrile as the calibrant, using Equation 6.2, where K_0 is the reduced mobility in units of $cm^2V^{-1}s^{-1}$ and t is the drift time of the calibrant and the unknown.

$$K_0^{unknown} = \frac{K_0^{calibrant} t^{calibrant}}{t^{unknown}} \qquad \text{Eq. 6.2}$$

The reduced mobilities calculated for TNT and RDX using both the direct injection and SBSE-IMS are shown in Table 6-1. For SBSE thermal desorption, the desorption time for analytes depends on temperature of the desorber and the flow rate of carrier gas. The geometric design of a desorber interface could potentially affect the time it takes an analyte to reach the detector. The results from Table 6-1 however, show very similar drift times and identical reduced mobilities for direct injection and SBSE desorption, indicating that not only is the desorption fast, but also very effective in terms of ion transfer into the IMS inlet.

Several studies were previously conducted on SBSE using the TDU for GC analysis where desorption times and temperature parameters were adjusted to achieve minimal carryover [259,260]. However, the kinetics of the desorption process of analytes from the stir bar has not been thoroughly explored. Although thermal desorption using the TDU is somewhat similar to the SBSE-IMS interface, the differences between the two techniques are significant. The desorption process using the TDU is not only significantly

	Direct Injecion		SBSE Desorption	
	Drift Time (ms)	Reduced Mobility (cm2V-1s-1)	Drift Time (ms)	Reduced Mobility (cm2V-1s-1)
TNT	12.365	1.454	12.360	1.454
RDX	12.927	1.391	12.923	1.391

Table 6-1. Comparison of drift time and reduced mobilities of TNT and RDX by direct injection IMS and SBSE-IMS.

longer that SBSE-IMS interface, but it also includes a cold trapping and re-concentration step to prevent band broadening in GC. Since the SBSE-IMS interface employs carrier gas at flow rates as high as 400 mL/min, less burden is placed on the thermal desorption and partitioning process, since the carrier gas removes analytes from the stir bar and desorption region quickly and exhaustively. In addition to the near elimination of carryover, the speed of the SBSE-IMS technique provides an interesting advantage in that it presents an insight into the desorption kinetics of the analytes by providing actual "live" snapshots of analytes desorbing from the stir bar coating.

Figure 6.5 depicts the live snapshots taken by the IMS of the desorbing process for TNT and RDX from the stir bar during the 60 second duration period. Initially there is no TNT or RDX present in the system. When the stir bar temperature is increased, the gas/coating partition coefficient decreases rapidly and the diffusion coefficient of analytes in the coating increases. As the gas flows through the chamber, analytes are removed from the outer most layer of the PDMS. As the desorption process penetrates deeper into the PDMS, layer, the amount of analytes desorbed increases. The desorption time that produces the maximum analyte response, defined as T_{Dmax} occurs at 25 seconds for TNT and 35 seconds for RDX. The 10 seconds difference in T_{Dmax} can be attributed to the large difference in boiling points of the two analytes. After the maximum desorption time points, the responses tail off as less analyte remains in the PDMS. Even though the responses do not appear to return to baseline after 60 seconds, complete desorption of the analytes do occur because no carryover is observed for subsequent desorptions.

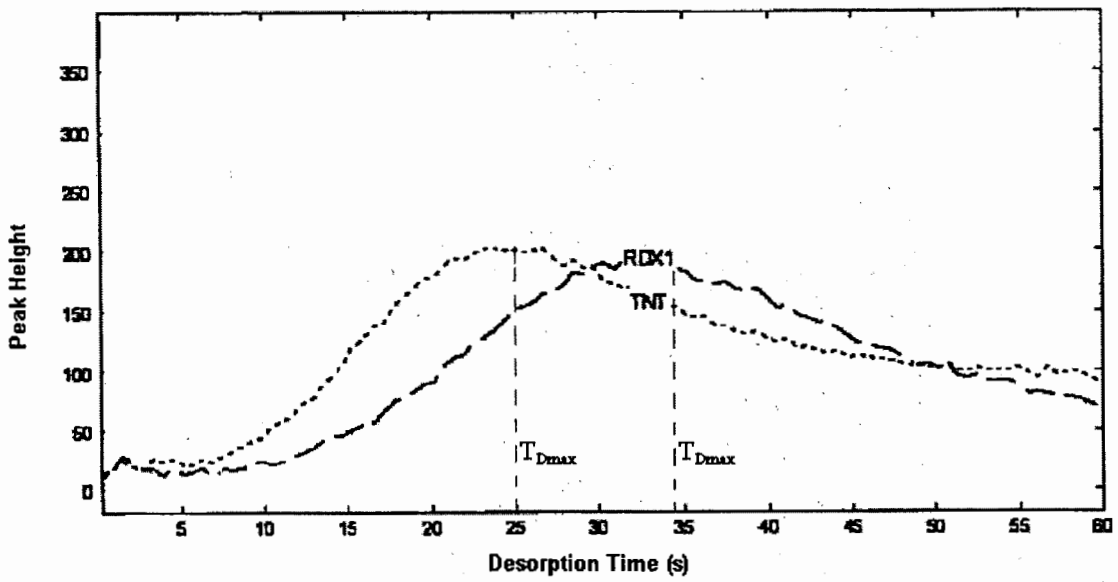


Figure 6.5. The snapshots of the live desorption profile of TNT and RDX desorbed directly on the SBSE-IMS interface at 280°C, 400 mL/min for 60 seconds.

The thermal desorption process of analytes from SPME has been previously described by Pawliszyn where the desorption time is independent on the distribution constant, K_{fg}, and depends on the thickness of the coating and also the diffusion coefficient of analytes [261]. The desorption process of analytes from SBSE can be achieved by the time given in Equation 6.2.

$$t \propto \frac{c}{D_f} \qquad \text{Eq. 2}$$

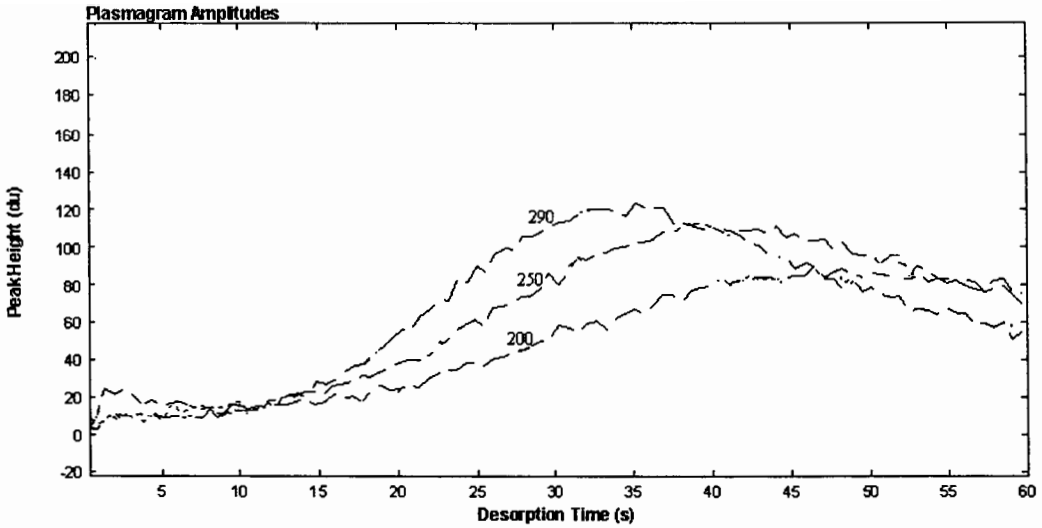
where t is the desorption time, c is the coating thickness on the stir bar and D_f is the diffusion coefficient of analyte.

In previous studies conducted using SBSE-GC, the complete removal of analytes from the stir bar has been challenging where minimization of carryover was only accomplished by increasing temperature and programming long desorption times [262,263]. In the SBSE-IMS analysis, no carryover was observed at or above temperatures of 250°C for 60 seconds desorption. Neither TNT nor RDX appeared in any of the blanks subsequently desorbed.

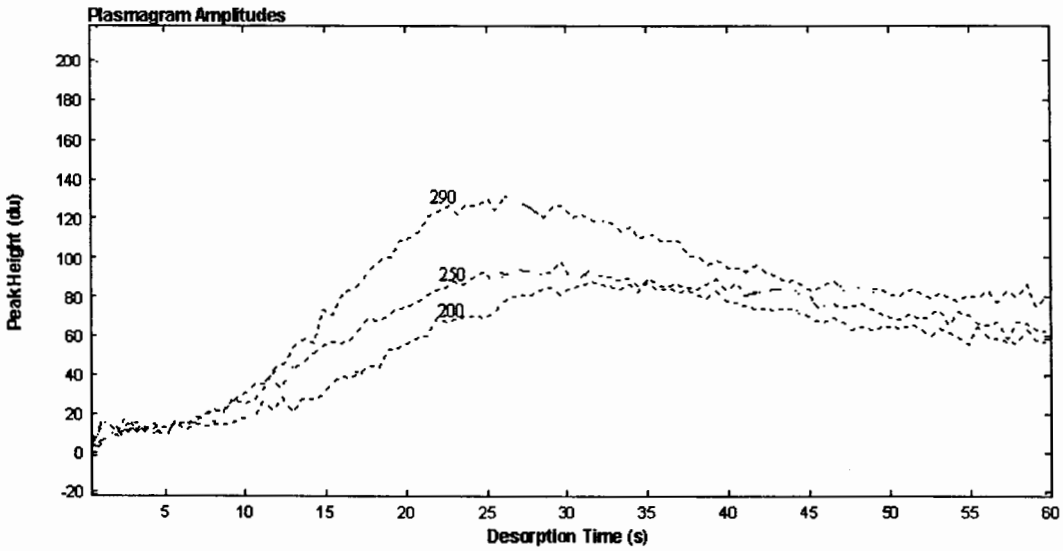
A temperature study on the desorption profile of TNT and RDX was conducted by desorbing the analytes at 200°C, 250°C, 270°C and 290°C, with all other parameters remaining constant. The T_{Dmax} results for TNT and RDX at the various desorption temperatures along with carryover information are shown in Table 6-2. The overlaid plasmagram profiles at 200°C, 250°C, and 290°C are shown in Figure 6.6. It can be seen that as the desorption temperature rises, the maximum peak height also increases, which

	Desorption Temperature (°C)	T_{Dmax} (s)	Carryover
TNT	200	35	5%
	250	30	0
	270	27	0
	290	25	0
RDX	200	50	7%
	250	42	0
	270	37	0
	290	35	0

Table 6-2. The T_{Dmax} and carryover results for TNT and RDX at desorption temperatures of 200°C, 250°C, 270°C and 290°C, with a flow rate of 400 mL/min for 60 seconds.



(A)



(B)

Figure 6.6. Overlays of the live desorption profile of (A) RDX and (B) TNT desorbed directly using the SBSE-IMS interface at 200°C, 250°C and 290°C, with a flow rate at 400 mL/min for 60 seconds.

enhances the sensitivity of the method. Another important observation from this study is the shifts in the time for maximum desorption, T_{Dmax}. As the temperature changes from 200°C to 290°C, the T_{Dmax} for both TNT and RDX become shorter indicating that as the temperature increases larger amounts of analytes are desorbed in a shorter period of time.

The relationship of T_{Dmax} vs. desorption temperature for both analytes shown graphically in Figure 6.7, and is determined to be linear. Although based on these observations, it would be ideal to run the SBSE-IMS interface at the highest possible temperature, all studies were conducted at 280 °C to preserve the integrity of the stir bar and also to reduce the baseline disturbances observed at higher temperatures.

According to Equation 6.2 and also on the thermal desorption principles previously developed for SPME, the desorption time is independent on the amount of analyte present in the coating. The relationship of T_{Dmax} vs. analye concentration was investigated by executing the method on spiked solutions of TNT from 0.001 μg/mL to 0.05 μg/mL and RDX from 0.01 μg/mL to 0.5 μg/mL.

The T_{Dmax} were obtained from the desorption profiles and plotted against their respective analyte concentrations. As seen in Figure 6.8, T_{Dmax} remains fairly constant over the concentration range investigated for both analytes confirming that the desorption rate for SBSE is independent on analye concentrations. No carryover was observed for these spiked samples.

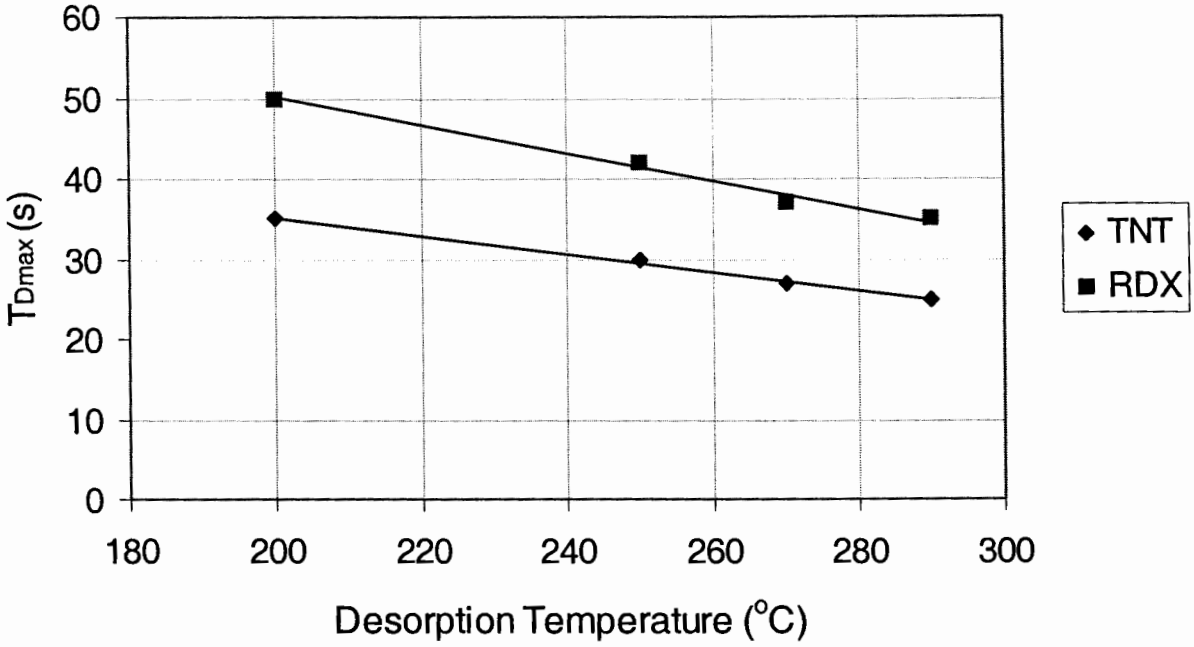


Figure 6.7. A plot showing the linear inverse relationship of T_{Dmax} vs. desorption temperature. Desorption carried out at 400 mL/min for 60 seconds.

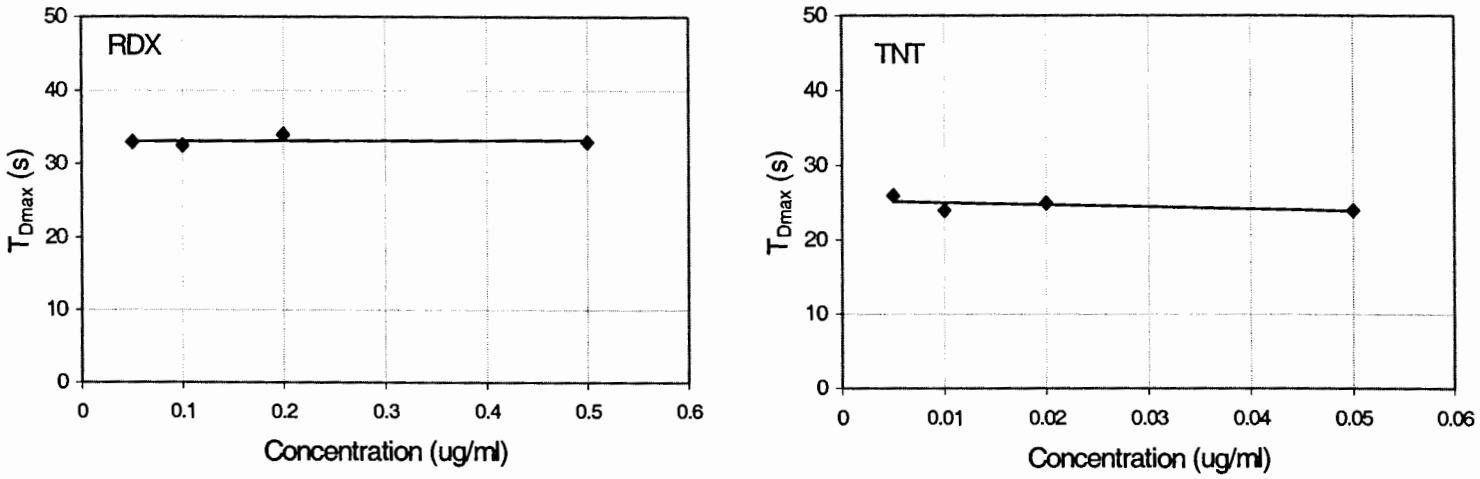


Figure 6.8. Effect of analyte concentration factor on the thermal desorption parameter, T_{Dmax} for RDX and TNT. Desorption at 280°C and 400 mL/min for 60 seconds.

The desorption process, which is kinetically controlled and measured using T_{Dmax} in this study, provides a useful tool in measuring the level of effectiveness of the desorption of analytes from the stir bar. The T_{Dmax} is dependent on analyte's properties such as boiling point and the diffusion coefficient between the PDMS phase and gas phase. Other experimental factors such as the thickness of the stir bar coating phase, desorber temperature, flow rates, position of the stir bar in the chamber could also affect the T_{Dmax} and the total desorption time. Generally, the shortest possible T_{Dmax} with no carryover is desirable for efficient analyte desorption.

The precision of the method, both in terms of drift time and peak response is important in the identification and quantitation of real sample analysis. Therefore the system reproducibility was evaluated for TNT and RDX by running five replicate samples by SBSE-IMS. The method precision for peak responses was 8.6% for TNT and 6.6% for RDX and the precision for drift times of both analytes were determined to be 0.1%. The results indicate that both peaks drift times and responses have good reproducibility with the SBSE-IMS interface. The limits of detection of the method was also evaluated and determined to be 1.5 ng/mL for RDX and 0.10 ng/mL for TNT. With appropriate matrix

interference studies using real explosives-contaminated water samples, the fast, solvent-less and portable method has the potential to be successful for explosives monitoring.

6.5 CONCLUSION

The fast removal of analytes from the stir bar provided excellent insights into the kinetically driven desorption process. The higher temperature not only provided shorter

desorption times, but also enhances the sensitivity of the technique by producing greater peak heights. The temperature desorption profiles generated by this technique can be used to optimize methods not only for SBSE-IMS, but also for SBSE-GC. The T_{Dmax}, which is an analyte-dependent parameter and also a function of temperature and flow rates provides a useful tool in optimizing desorption conditions, because a short desorption and elimination of carryover is critical for the technique to be successful.

The coupling of SBSE with IMS creates a straightforward system with high sensitivity, speed, portability and a means of detecting explosives in water samples. The addition of an extraction step, such as SBSE provides additional selectivity for the technique in terms of reducing matrix interferences, which is critical for IMS detection. The excellent performance of the interface developed for the method is demonstrated by the fast and effective desorption time for analytes, with no carryover, and good reproducibility. The effectiveness of the desorption is further established by the similarity of the drift times and peak shape characteristics of analytes when compared to direct injection. The pre-concentration characteristics of SBSE also complements and enhance the intrinsic sensitivity IMS. Using a more polar SBSE coating phase can significantly increase the limit of detection for both analytes, especially the more polar RDX. Unfortunately the only phase that is currently available commercially is the non-polar PDMS. The excellent reproducibility for drift time, which is important of identification, along with method precision of 8.6% for TNT and 6.6% for RDX and limits of detection in the low part-per-billion levels render the SBSE-IMS method very promising for the detection of explosives in water and other routine field applications.

Final Conclusion

This research project has successfully shown that the sorptive extraction techniques solid phase microextraction and stir bar sorptive extraction can be used effectively with ion mobility spectrometry to detect and quantitate low levels of analytes in various sample matrices. The extraction techniques not only enhance the sensitivity and selectivity of IMS, but also complement the instrumentation's solventless, fast, portable and field capability characteristics.

In the first phase of the project it was shown that SPME-IMS does not require elaborate sample preparation or chromatographic separation and provides a fast, sensitive and reliable method for detecting and quantifying ephedrine in a biological matrix. Although no interference by coexisting substances was observed, confirmation analysis by GC-MS should be performed for IMS-positive samples. The linearity for ephedrine response, along with a detection limit of 0.05 μg/mL, reproducibility of 5% and good recoveries make the method very satisfactory. The limit of detection is well below the cut-off limit specified by the IOC medical commission

The chemical ionization mass spectra of ephedrine also compared well with the IMS fragmentation pattern. The three major ions produced by IMS match that of the mass spectra with respect to drift times and molecular weight indicating that structural information can be obtained from IMS plasmagrams. It was deduced that the IMS fragmentation spectra can be used to give additional evidence for the presence of ephedrine in a sample. The developed method is very practical for forensic applications because of short analysis time, minimization of carry-over, fragmentation characteristics,

and field-analysis capabilities. However, further evaluation of this technique against well-understood and established methods is necessary before SPME-IMS can be solely relied upon for clinical or forensic measurements.

The SPME-IMS technique was taken a step further and developed for the simultaneous detection, separation and quantitation of methylparaben, ethylparaben, propylparaben and butylparaben in pharmaceutical cream, lotion, solution and ointment samples. The separation characteristics of the ions were explored and optimized with respect to temperature and carrier gas flow rates. The drift time behaviors of the paraben ions were also evaluated with respect to drift tube temperature and flow rates. The stable reduced mobilities determined from the temperature studies indicated that the paraben ions observed in the plasmagrams are most likely parent ions and not produced from ion clusters or fragmentation.

An internal standard, benzylparaben, was used to optimize the paraben quantitation. This calibration technique proved to be significantly more effective than external standards, both in terms of reproducibility and recovery. The quantitation of preservatives in the six samples using the established HPLC method indicated good agreement when compared to the SPME-IMS methods. The percent differences observed between the two methods ranged from 1.4% for MP in Lotion A to 12.5% in Ointment A. The larger discrepancies between the two methods were observed in samples that contained high levels of interfering compounds with respect to parabens, which resulted in ion interferences.

The method reproducibility of less than 8%, linear range of 3 orders of magnitude and sensitivities of less than 10 ng/mL rendered the SPME-MS method very attractive and promising for routine applications. The excellent separation of the four analytes and internal standard with resolution ≥ 1 and the theoretical plates $>10{,}000$ for all parabens in less than 18 ms is also remarkable for an IMS method.

The main advantages of the SPME-IMS method over HPLC include the reduction of labor-intensive extraction steps, elimination of solvent consumption in extraction and mobile phase preparation, and shortened analysis time from hours to minutes. The good selectivity, separation capability, limit of detection, linear range, reproducibility, and recovery render the SPME-MS method attractive and more advantageous over traditional techniques.

In the final phase of the project, a new technique, SBSE-IMS is shown to be effective using thermal desorption for the detection of TNT and RDX in water samples. In addition to all of the advantages provided by SPME, SBSE possesses the ability to increase the sensitivity of a method by several orders or magnitude. The SBSE-IMS method was developed and optimized in terms of extraction efficiency and ion separation, ions fragmentation patterns, thermal desorption and carryover effects.

The drift times characteristics, diffusion coefficients, and experimental parameters on the kinetics of thermal desorption were also explored. Since the separation and detection is so fast using IMS, it was possible to obtain live snapshots of the desorption process. These profiles provide a useful insight into the kinetics of thermal desorption utilizing the newly developed parameter, T_{Dmax}. The new interface was shown to work effectively using its temperature control and very high linear flow rates to produce short

T_{Dmax} and eliminate carryover. Higher temperatures not only provided shorter desorption times, but also enhanced the sensitivity of the technique by producing larger peak heights.

The T_{Dmax}, which is function of stir bar coating thickness and analyte diffusion coefficient provides a useful tool to optimize desorption conditions, because a fast desorption and elimination of carryover is critical for any method. Complete desorption was achieved in 60 seconds using a temperature of 280°C and a flow rate of 400 mL/min. The desorption efficiency of the SBSE-IMS technique, reflected by the fast desorption time is significantly shorter than TDU desorption carried out for GC analysis.

The reproducibility of the SBSE-IMS method was evaluated and peak responses were determined to be 8.6% for TNT and 6.6% for RDX. The drift time precision for both analytes were determined to be 0.1%. The limits of detection were also determined to be 1.5 ng/mL for RDX and 0.1 ng/mL for TNT.

The SBSE-IMS method creates a straightforward solventless system with high sensitivity, speed, portability and a means of detecting analytes in real samples. The addition of SBSE to IMS not only improves the sensitivity but also provides additional selectivity for the technique in terms of reducing matrix interferences. The effectiveness of the interfacing technique is further illustrated by the similarity of the drift times and peak shape characteristics of analytes when compared to direct injection.

The results presented in this research are very encouraging, supporting the future application of SPME-IMS and SBSE-IMS for the low level detection of analytes. The techniques have the potential to be tremendously successful in analytical field applications such as the determination of illicit drugs, forensics, pharmaceuticals and

environmental toxins determination. The sorptive extraction step prior to IMS analysis circumvents the major limitation of IMS, specifically ion competition and suppression which result in loss of specificity and low resolution.

The next logical extension of this work would be to apply the techniques to real applications with additional specificity, recoveries and comparability studies. The use of mobility measurements and fragmentation patterns to probe molecular and structural information of analytes is also not yet a mature field in IMS studies. The sorptive extraction techniques can also be used to perform "on-fiber" and "on-stir bar" derivatization reactions by adding functional groups to analytes thereby promoting ionization to facilitate the analysis of compounds that would otherwise be unfeasible by IMS. Derivatizations can also be used to enhance the sensitivity of the methods.

The novel technique of thermal desorption SBSE-IMS is shown to be effective for TNT and RDX, and has the potential to be enormously successful for the ultra trace detection of analytes with unprecedented sensitivity. The speed of the IMS also permits the elucidation of the kinetics of thermal desorption from a sorptive phase as shown in Chapter 6. These studies using live snapshots are not possible using traditional chromatographic techniques because the analysis time is so much longer than the desorption time. Further work in this area would be to investigate parameters such as coating type, coating thickness, as well as analytes molecular weight, diffusion coefficient and vapor pressure on the T_{Dmax} and carryover effects.

Sorptive extraction techniques coupled to IMS is a new and emerging method that possesses a robust model of a standardized test method with the necessary speed, sample

throughput, solvent elimination, and high sensitivity rendering the methods capable of moving into the mainstream of field analytical chemistry.

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